

Applicants: Zhongyi Li et al.

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**Exhibit 1**



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(71) Applicants (for all designated States except US): COMMON-WEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, Australian Capital Territory 2601 (AU). GOODMAN FIELDER LIMITED [AU/AU]; Level 42 Grosvenor Place, Sydney, New South Wales 2000 (AU). GROUPE LIMAGRAIN PACIFIC PTY LTD [AU/AU]; Level 31, 1 O'Connell Street, Sydney, New South Wales 2000 (AU).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MORELL, Matthew [AU/AU]; 33 Wangara Street, Aranda, Australian Capital Territory 2614 (AU). LI, Zhongyi [AU/AU]; 63 Campaspe Circuit, Kaleen, Australian Capital Territory 2617 (AU). RAHMAN, Sadequr [AU/AU]; 46 Scarlett Street, Melba, Australian Capital Territory 2615 (AU). APPELS, Rudolph [AU/AU]; 40 Gingara Street, Aranda, Australian Capital Territory 2614 (AU).

(74) Agents: OLIVE, Mark, R. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, Victoria 3000 (AU).

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(54) Title: NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR

(57) Abstract

The present invention provides isolated nucleic acid molecules encoding wheat starch synthases, and probes and primers derived therefrom, which are useful in the modification of plant starch content and/or composition, and for screening plant lines to determine the presence of natural and/or induced mutations in starch synthase genes which affect starch content and/or composition. More particularly, the isolated nucleic acid molecules of the present invention further provide for the screening-assisted breeding of plants having desirable starch content and/or composition, in addition to providing for the direct genetic manipulation of plant starch content and/or composition.

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## NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES THEREFOR

### FIELD OF THE INVENTION

5 The present invention relates generally to isolated nucleic acid molecules encoding wheat starch synthase enzymes and more particularly, to isolated nucleic acid molecules that encode wheat SSII and SSIII enzyme activities. The isolated nucleic acid molecules provide the means for modifying starch content and composition in plants, for example the ratio of amylose:amylopectin in the starch granule of the  
10 endosperm during the grain-filling phase of endosperm development. The isolated nucleic acid molecules of the present invention also provide the means for screening plant lines to determine the presence of natural and/or induced mutations in starch synthase genes which affect starch content and/or composition. The isolated nucleic acid molecules of the present invention further provide for the screening-assisted  
15 breeding of plants having desirable starch content and/or composition, in addition to providing for the direct genetic manipulation of plant starch content and/or composition.

### GENERAL

Bibliographic details of the publications numerically referred to in this specification are  
20 collected at the end of the description. Reference herein to any published document is not to be taken as an indication or admission that any such published document is part of the common general knowledge or background information of a skilled worker in the relevant field.

25 This specification contains nucleotide and amino acid sequence information (SEQ ID NOS:) prepared using the programme PatentIn Version 2.0, presented herein at the end of the specification. Each nucleotide or amino acid sequence is identified in the sequence listing by the numeric indicator <210> followed by the sequence identifier (e.g. <210>1, <210>2, etc). The length, type of sequence (DNA, protein (PRT), etc)  
30 and source organism for each nucleotide or amino acid sequence are indicated by information provided in the numeric indicator fields <211>, <212> and <213>.



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respectively. Nucleotide and amino acid sequences (SEQ ID NOs:) referred to in the specification are defined by the information provided in numeric indicator field <400> followed by the sequence identifier (eg. SEQ ID NO: 1 is <400>1, etc).

- 5 The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W  
10 represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.
- 15 The designations for naturally-occurring amino acid residues referred to herein are set forth in Table 1. The designations for a non-limiting set of non-naturally-occurring amino acids is listed in Table 2.

As used herein the term "derived from" shall be taken to indicate that a specified  
20 integer may be obtained from a particular source albeit not necessarily directly from that source.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to  
25 imply the inclusion of a stated step or element or integer or group of steps or elements or integers but not the exclusion of any other step or element or integer or group of steps or elements or integers.

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TABLE 1

Amino Acid	Three-letter Code	One-letter Code
5 Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
10 Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
15 Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
20 Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
25 Aspartate/glutamate	Baa	B
Asparagine/glutamine		
Any amino acid as above	Xaa	X

TABLE 2

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5				
	$\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
	aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
			L-N-methylaspartic acid	Nmasp
10	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
			L-N-methylglutamic acid	Nmglu
	cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
25	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva

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	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgab
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
5	D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methyllleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
	D- $\alpha$ -methylllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- $\alpha$ -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- $\alpha$ -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

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	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl) glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
5	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl) glycine	Nhis
	D-N-methyllleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
10	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
15	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
20	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
	L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methy lasparagine	Masn
	L- $\alpha$ -methy laspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
25	L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- $\alpha$ -methylglutamine	Mglu	L- $\alpha$ -methylglutamate	Mglu
	L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomo phenylalanine	Mhphe
	L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
30	L- $\alpha$ -methyllleucine	Mleu	L- $\alpha$ -methyllysine	Mlys

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L- $\alpha$ -methylnorleucine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
5 L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
L- $\alpha$ -methylvaline	Mval	L-N-methylhomo	
		phenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbonylmethyl)glycine	Nnbhm	carbonylmethyl)glycine	Nnbhe
10 1-carboxy-1-(2,2-diphenyl-			
ethylamino)cyclopropane	Nmbc		

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Those skilled in the art will appreciate that the invention described herein is susceptible  
 15 to variations and modifications other than those specifically described. It is to be  
 understood that the invention includes all such variations and modifications. The  
 invention also includes all of the steps, features, compositions and compounds  
 referred to or indicated in this specification, individually or collectively, and any and all  
 combinations or any two or more of said steps or features.

20

The present invention is not to be limited in scope by the specific embodiments  
 described herein, which are intended for the purposes of exemplification only.  
 Functionally-equivalent products, compositions and methods are clearly within the  
 scope of the invention, as described herein.

25

## BACKGROUND TO THE INVENTION

The biosynthesis of the starch granule is a complex process which involves the action  
 of an array of isoforms of enzymes involved in the starch biosynthesis. Following the  
 formation of glucose-1-phosphate, the enzyme activities required for the synthesis of  
 30 granular starch include ADP glucose pyrophosphorylase (EC 2.7.7.27), starch  
 synthases (EC 2.4.1.21), branching enzymes (EC 2.4.1.18) and debranching enzymes

(EC 3.2.1.41 and EC 3.2.1.68) (Mouille *et al.*, 1996). Plants contain isozymes of each of these activities, and the definition of these isoforms and their roles has been conducted through investigation of the properties of the suite of soluble enzymes found in the stroma of the plastid, analysis of the proteins entrapped within the matrix of the starch granule, and mutational studies to identify genes and define linkages between individual genes and their specific roles.

Starch synthases extend regions of  $\alpha$ -1,4 glucan through the transfer of the glucosyl moiety of ADPglucose to the non-reducing end of a pre-existing  $\alpha$ -1,4 glucan. In addition to GBSS, 3 other classes of starch synthase have been identified in plants, SSI (wheat, Li *et al.*, 1999 and GenBank Accession No. U48227; rice, Baba *et al.*, 1993; potato, Genbank Accession No. STSTASYNT), SSII (pea, Dry *et al.* 1992; potato, Edwards *et al.*, 1995; maize, Harn *et al.* 1998 and GenBank Accession No. U66377) and SSIII (potato, Abel *et al.*, 1996; maize, Gao *et al.*, 1998). In the cereals, the most comprehensively studied species is maize, where in addition to GBSS, cDNAs encoding SSI, SSIIa, and SSIIb have been isolated, and both cDNA and genomic clones for *dull1* have been characterised (Knight *et al.*, 1998; Harn *et al.*, 1998; Gao *et al.*, 1998). In maize, the product of the *du1* gene is known as maize SSII, however this gene is the homologue of potato SSIII.

20

The proteins within the matrix of the wheat starch granule have been extensively studied (Denyer *et al.*, 1995; Rahman *et al.*, 1995; Takaoka *et al.*, 1997; Yamamori and Endo, 1996) and 60, 75, 85, 100, 104 and 105 kDa protein bands can be visualised following SDS-PAGE. The predominant 60 kDa protein is exclusively granule-bound and is analogous to the "waxy" granule bound starch synthase (GBSS) gene in maize (Rahman *et al.*, 1995). The combination of three null alleles for this enzyme from each of the wheat genomes (Nakamura *et al.*, 1995) results in the amylose-free "waxy" phenotype found in other species. The 75 kDa starch synthase I (wSSI) is found in both the granule and the soluble fraction of wheat endosperm (Denyer *et al.*, 1995; Li *et al.*, 1999) and has been assigned to chromosomes 7A, 7B and 7D (Yamamori and Endo, 1996; Li *et al.*, 1999). The 85 kDa band contains a

30



class II branching enzyme and an unidentified polypeptide (Rahman *et al.*, 1995). The 100, 104 and 105 kDa proteins of the wheat starch granule (designated Sgp-B1, Sgp-D1 and Sgp-A1 by Yamamori and Endo, 1996) have been shown to be encoded by a homeologous set of genes on the short arm of chromosome 7B, 7A and 7D 5 respectively (Yamamori and Endo, 1996; Takaoka *et al.*, 1997). Denyer *et al.* (1995) concluded on the basis of enzyme activity assays that these proteins were also starch synthases. These genes are referred to hereinafter as the "wheat SSII genes".

While GBSS has been established to be essential for amylose synthesis, the remaining 10 starch synthases are thought to be primarily responsible for the elongation of amylopectin chains, although this does not preclude them from also having non-essential roles in amylose biosynthesis. Differences in kinetic properties between isoforms, and the analysis of mutants lacking various isoforms, suggests that each isoenzyme contributes to the extension of specific subsets of the available non- 15 reducing ends.

## SUMMARY OF THE INVENTION

The production of plants that produce improved starches that are modified for particular end-use applications, such as, for example, starches having high or low 20 amylose:amylopectin ratios, requires the availability of genes encoding the various starch synthase isoforms. Because of species-specific codon usages, and variations in the kinetic parameters of the starch synthase isoforms between species, the production of modified starches may require the use of genes derived from particular species.

25

Furthermore, the screening-assisted breeding of plants having desirable starch content and/or composition requires specific gene sequences to be provided that can be used to distinguish between different homeologous genes encoding the various isoforms of wheat starch synthases, such as, for example, to identify and distinguish between 30 naturally-occurring variant gene sequences. It is a particular object of the present invention to provide gene sequences to facilitate the screening-assisted selection of

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wheat plants having starch traits which are associated with the presence and/or expression of one or more wheat SSI and/or SSIII genes.

Accordingly, the present invention provides isolated nucleotide sequences encoding  
5 the wheat SSII (i.e. wSSII) and wheat SSIII (i.e. wSSIII) isoenzymes, and DNA markers derived therefrom. The present invention further facilitates the production of transformed plants carrying these nucleotide sequences.

More particularly, the present invention provides isolated nucleic acid molecules  
10 encoding the 100, 104 and 105 kDa SSII (Sgp-1) polypeptides of the wheat starch granule matrix, as determined using the SDS/PAGE system of Rahman *et al.* (1995), which polypeptides are equivalent to the 100, 108 and 115 kDa polypeptides described by Yamamori and Endo (1996).

15 The present invention further provides isolated nucleic acid molecules encoding the soluble *dull1*-type wheat starch synthase III polypeptide. Analysis of the polypeptides encoded by these nucleic acid molecules reveals several consensus amino acid sequence motifs that are highly conserved in wheat starch synthase isoenzymes, in addition to isoenzyme-specific sequences, which sequences possess utility in isolating  
20 related starch synthase-encoding sequences and in assaying plants for their expression of one or more starch synthase isoenzymes.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is  
25 complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at  
30 least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

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(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

5 (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- 10 (a) KVGGLGDVWTS (SEQ ID NO: 39);  
(b) GHTVEVILPKY (SEQ ID NO: 40);  
(c) HDWSSAPVAWLYKEHY (SEQ ID NO: 41);  
(d) GILNGIDPDIDWDPYTD (SEQ ID NO: 42);  
(e) DVPIVGIITRLTAQKG (SEQ ID NO: 43);  
(f) NGQVLLGSA (SEQ ID NO: 44);  
15 (g) AGSDFIIVPSIFPCGLTQLVAMRYGS (SEQ ID NO: 45); and  
(h) TGGLVDTV (SEQ ID NO: 46);

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

20 (iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- 25 (a) KTGGLGDVAGA (SEQ ID NO: 47);  
(b) GHRVMVVVPY (SEQ ID NO: 48);  
(c) NDWHTALLPVYLKAYY (SEQ ID NO: 49);  
(d) GIVNGIDNMEWNPEVD (SEQ ID NO: 50);  
(e) DVPLLGFGRDLGQKG (SEQ ID NO: 51);  
(f) DVQLVMLGTG (SEQ ID NO: 52);  
30 (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO: 53); and  
(h) VGG(V/L)RDTV (SEQ ID NO: 54);

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wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

5 In a preferred embodiment, the isolated nucleic acid molecule encodes a starch synthase polypeptide, protein or enzyme having at least about 90% amino acid sequence identity to any one of SEQ ID NOS: 2, 4, 6, 8 or 10, more preferably having at least about 95% or about 97% or about 99% identity to any one of said amino acid sequences.

10

In an alternative embodiment, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

- (a) GHTVEVILPKY;
- 15 (b) HDWSSAPVAWLYKEHY;
- (c) DVPIVGIITRLTAQKG;
- (d) NGQVVLLGSA;
- (e) AGSDFIIVPSIFPCGLTQLVAMRYGS;
- (f) TGGLVDTV;
- 20 (g) GIVNGIDNMEWNPEVD; and
- (h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

in an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme  
25 molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence having at least about 85% nucleotide sequence identity to any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37 or 38 or a complementary nucleotide sequence thereto.

30 In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37 or 38,

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or is at least about 90% identical, more preferably at least about 95% or 97% or 99% identical to all or a protein-encoding part thereof.

In an alternative embodiment, the present invention provides an isolated nucleic acid  
5 molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence that is capable of hybridising under at least moderate stringency hybridisation conditions to at least about 30 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37 or 38, or a complementary  
10 nucleotide sequence thereto.

A second aspect of the present invention provides a method of isolating a nucleic acid molecule that encodes a starch synthase polypeptide, protein or enzyme described *supra*, said method comprising:

- 15 (i) hybridising a probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37 or 38, or a complementary nucleotide sequence thereto to single-stranded or double-stranded mRNA, cDNA or genomic DNA; and  
(ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting  
20 means.

Preferably, the detecting means is a reporter molecule covalently attached to the probe or primer molecule or alternatively, a polymerase chain reaction format. Accordingly, the present invention clearly extends to the use of the nucleic acid molecules provided  
25 herein to isolate related starch synthase-encoding sequences using standard hybridisation and/or polymerase chain reaction techniques.

A third aspect of the invention provides an isolated probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3,  
30 5, 7, 9, 11-16, 37 or 38, or a complementary nucleotide sequence thereto.

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Preferably, the probe or primer comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 25 to 34.

A fourth aspect of the present invention is directed to an isolated or recombinant starch  
5 synthase polypeptide, protein or enzyme, preferably substantially free of conspecific or non-specific proteins, which comprises an amino acid sequence selected from the following:

(i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or  
functional subunit thereof which comprises an amino acid sequence which is at  
10 least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;

(ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or  
functional subunit thereof which comprises an amino acid sequence which is at  
15 least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional  
subunit thereof which comprises a conserved amino acid sequence having at  
least 25% identity to an amino acid sequence selected from the group  
consisting of:

- 20 (a) KVGGLGDVVTs;  
(b) GHTVEVILPKY;  
(c) HDWSSAPVAWLYKEHY;  
(d) GILNGIDPDIWDPYTD;  
(e) DVPIVGIITRLTAQKG;  
25 (f) NGQVLLGSA;  
(g) AGSDFIIVPSIFEPCGLTQLVAMRYGS; and  
(h) TGGLVDTV

wherein said wheat starch synthase polypeptide further comprises an amino  
acid sequence having at least about 85% identity overall to an amino acid  
30 sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional



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subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KTGGLGDVAGA;
- 5 (b) GHRVMVVVPRY;
- (c) NDWHTALLPVYLKAYY;
- (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFGRLDGQKG;
- (f) DVQLVMLGTG;
- 10 (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (h) VGG(V/L)RDTV

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

15

The present invention clearly encompasses the mature protein region of a wheat starch synthase polypeptide which is obtained by removal of the N-terminal transit peptide sequence.

- 20 A further aspect of the invention provides a method of assaying for the presence or absence of a starch synthase isoenzyme or the copy number of a gene encoding same in a plant, comprising contacting a biological sample derived from said plant with an isolated nucleic acid molecule derived from any one of SEQ ID NOS 1, 3, 5, 7, 9, 11-16, 37 or 38, or any one of SEQ ID NOS: 25 to 34, or a complementary nucleotide
- 25 sequence thereto for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

The detection means according to this aspect of the invention is any nucleic acid based hybridisation or amplification reaction.

30

A further aspect of the present invention utilises the above-mentioned assay method



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in the breeding and/or selection of plants which express or do not express particular starch synthase isoenzymes or alternatively, which express a particular starch synthase isoenzyme at a particular level in one or more plant tissues. This aspect clearly extends to the selection of transformed plant material which contains one or  
5 more of the isolated nucleic acid molecules of the present invention.

A further aspect of the present invention provides a method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing therein a sense molecule, antisense molecule, ribozyme  
10 molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified. This aspect of the invention clearly extends to the  
15 introduction of the sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule to isolated plant cells, tissues or organs or organelles by cell fusion or transgenic means and the regeneration of intact plants therefrom.

20 A further aspect of the present invention provides an isolated promoter that is operable in the endosperm of a monocotyledonous plant cell, tissue or organ, and preferably in the endosperm of a monocotyledonous plant cell, tissue or organ. For example, the HMG promoter from wheat, or the maize zein gene promoter are particularly preferred, as is the promoter derived from a starch synthase gene of the present invention, such  
25 as a promoter that is linked *in vivo* to any one of SEQ ID NOS 1, 3, 5, 7, 9,11-16, 37 or 38, or a complementary nucleotide sequence thereto.

A still further aspect of the present invention contemplates a transgenic plant comprising an introduced sense molecule, antisense molecule, ribozyme molecule, co-  
30 suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9,11-16,

37 or 38, or a complementary nucleotide sequence thereto or a genetic construct comprising same, and to plant propagules, cells, tissues, organs or plant parts derived from said transgenic plant that also carry the introduced molecule(s).

## 5 BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a copy of a photographic representation showing the distribution of wheat endosperm starch synthases between the starch granule and soluble fractions. Lane 1, SDS-PAGE of wheat endosperm starch granule proteins revealed by silver staining; lanes 2-7, immunoblot of wheat endosperm soluble phase and starch granule proteins separated by SDS-PAGE from various developmental stages and probed with an anti- (wheat wSSII peptide) monoclonal antibody. Lanes 2-4 contain proteins from the soluble fraction of wheat endosperm at 15 days post anthesis (Lane 2); 20 days post anthesis (Lane 3); and at 25 days post anthesis (Lane 4). Lanes 5-7 contain proteins from the starch granule of wheat endosperm at 15 days post anthesis (Lane 5); 20 days post anthesis (Lane 6); and at 25 days post anthesis (Lane 7).

**Figure 2** is a copy of a schematic representation comparing the nucleotide sequences of cDNA clones designated wSSIIA, wSSIIB and wSSIID, encoding the starch synthase II polypeptides from wheat, using the PILEUP programme of Devereaux *et al.* (1984).

**Figure 3** is a copy of a schematic representation comparing the deduced amino acid sequences of starch synthase II from wheat (wSSIIA, wSSIIB and wSSIID), maize (maize SSIIa and maize SSIIb; Harn *et al.*, 1998), pea (pea SSII; Dry *et al.*, 1992) and potato (potato SSII; van der Leij *et al.*, 1991). Identical amino acid residues among each of these sequences are indicated below the sequences with "\*". The alignments of maize SSIIa with maize SSIIb, and pea SSII and potato SSII are essentially as described in Harn *et al.* (1998) and Edwards *et al.* (1995). All sequences are aligned to position the transit peptide cleavage site below the arrow (↓) between residues 59 and 60 of the wSSIIA sequence. The wSSIIp1 sequence, the sequence of SGP-B1 (peptide3), and of eight conserved regions are annotated and underlined.

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**Figure 4** is a copy of a photographic representation of a northern blot showing the expression of wheat wSSII mRNA in wheat plants. Total RNAs were isolated from leaves pre-anthesis florets and endosperm of the wheat cultivar "Gabo", grown under a photoperiod comprising 16 hours daylength, and at 18 °C during the day, and at 13 °C during the night cycle, and probed with the wSSIIp2 DNA fragment. The source of each RNA is indicated at the top of the Figure as follows: Lane 1, leaf; Lane 2, pre-anthesis florets; Lanes 3-11, endosperm at: 4 days post-anthesis (Lane 3); 6 days post-anthesis (Lane 4); 8 days post-anthesis (Lane 5); 10 days post-anthesis (Lane 6); 12 days post-anthesis (Lane 7); 15 days post-anthesis (Lane 8); 18 days post-anthesis (Lane 9); 21 days post-anthesis (Lane 10); and 25 days post-anthesis (Lane 11).

**Figure 5** is a copy of a photographic representation showing the localization of wheat starch synthase II genes on the wheat genome by PCR, using the primers sslIc, sslId and sslIe in the amplification reaction. The nullisomic-tetrasomic genomic DNA of wheat cv. Chinese Spring was used as template DNA. Lane D, *Triticum tauschii*; Lane AB, Accession line N7DT7B having no 7D chromosome and four copies of the 7B chromosome; Lane AD, Accession line N7BT7A having no 7B chromosome and four copies of the 7A chromosome; Lane BD, Accession line N7AT7B having no 7A chromosome and four copies of the 7B chromosome; Lane ABD, wheat cv. Chinese Spring. PCR products derived from each cDNA clone are labelled. The results indicate that the cDNA clones, wSSII B, wSSII A and wSSII D are derived from the B-, A- and D-genomes of wheat, respectively.

**Figure 6** is a schematic representation showing the organisation of introns (lines) and exons (boxes) in the wheat SSII gene shown in SEQ ID NO: 37. The scale (bases), relative to the nucleotide sequence set forth in SEQ ID NO: 37, is provided at the bottom of the figure.

**Figure 7** is a schematic representation comparing the deduced amino acid Sequences of the maize, potato and wheat SSIII polypeptides.

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**Figure 8** is a copy of a photographic representation showing the expression of wheat wSSIII mRNA in wheat. Total RNAs were isolated from the endosperm of the wheat cultivars Wyuna (Panel a) and Gabo (Panel b) leaves pre-anthesis florets and endosperm of the wheat cultivar "Gabo", grown under a photoperiod comprising 16 hours daylength, and at 18 °C during the day cycle, and at 13 °C during the night cycle, and probed with the wSSIIIp1 DNA fragment derived from wSSIII.B3 cDNA. The source of each RNA is indicated at the top of the Figure as follows: Lane 1, endosperm at: 4 days post-anthesis; Lane 2, endosperm at 6 days post-anthesis; Lane 4, endosperm at 8 days post-anthesis; Lane 4, endosperm at 10 days post-anthesis; 10 Lane 5, endosperm at 12 days post-anthesis; Lane 6, endosperm at 15 days post-anthesis; Lane 7, endosperm at 18 days post-anthesis; Lane 8, endosperm at 21 days post-anthesis; Lane 9, endosperm at 25 days post-anthesis; and Lane 10, endosperm at 31 days post-anthesis (Panel a only). In panel (c), L refers to leaf RNA, and P refers to RNA from pre-anthesis florets derived from the cultivar Gabo.

15

**Figure 9** is a schematic representation showing the position of conserved amino acid sequences within four wheat starch synthase proteins. The eight highly-conserved regions between the wheat starch synthase polypeptides are underlined and annotated at the top of each group of amino acid sequences. The sequences included in the 20 alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (wGBSS; Yan *et al.*, 1999); wheat SSI (wSS1; Li *et al.*, 1999); wheat SSII (wSS2; SEQ ID NO: 4); and wheat SSIII (wSS3; SEQ ID NO: 8).

**Figure 10** is a schematic representation showing the relationships between the 25 primary amino acid sequences of starch synthases (SS) and glycogen synthase of *E. coli* (GS). The dendrogram was generated by the program PILEUP (Devereaux *et al.*, 1984). The amino acid sequences used for the analysis are those of the wheat SSIIA, wheat SSIIIB, wheat SSIIID, and wheat SSIII polypeptides of the present invention compared to the deduced amino acid sequences of wheat GBSS (Clark *et al.*, 1991), 30 wheat SSI (Li *et al.*, 1999), rice GBSS (Okagaki, 1992), rice SSI (Baba *et al.*, 1993), maize GBSS (Kloesgen *et al.*, 1986), maize SSI (Knight *et al.*, 1998), maize SSIIa and

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maize SSIIb (Harn *et al.*, 1998), maize SSIII (Gao *et al.*, 1998), pea GBSS (Dry *et al.*, 1992), pea SSII (Dry *et al.*, 1992), potato GBSS (van der Leij *et al.*, 1991), potato SSI (Genbank accession number: STSTASYNT), potato SSII (Edwards *et al.*, 1995), potato SSIII (Abel *et al.*, 1996), and *E. coli* glycogen synthase (GS) (Kumar *et al.*, 1986). Five groups of enzymes included in the alignment are granule-bound starch synthase (GBSS), starch synthase-I (SSI), starch synthase-II (SSII), starch synthase-III (SSIII) and glycogen synthase (GS).

Figure 11 is a schematic representation showing the position of conserved regions within cereal starch synthase genes. Comparisons of cereal starch synthases were made based on their deduced amino acid sequences and 8 conserved regions identified. Conserved regions are shown in bold and transit peptides (where defined) in grey. The sequences included in the alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (Ainsworth *et al.*, 1993); wheat SSI (Li *et al.*, 1999); maize SSIIa (Harn *et al.*, 1998); and maize dull-1 (Gao *et al.*, 1998).

Figure 12 is a copy of a schematic representation of a gene map showing the alignment of fragments 1 to 6 of the genomic SSIII gene (lower line) with the corresponding SSIII cDNA clone (upper line). Raised regions in the genomic clone fragments (lower line) represent protein-encoding regions of the gene.

Figure 13 is a schematic representation showing the organisation of introns (lines) and exons (boxes) in the wheat SSIII gene shown in SEQ ID NO: 38. The scale (bases), relative to the nucleotide sequence set forth in SEQ ID NO: 38, is provided at the bottom of the figure.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

One aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes, or is complementary to a nucleic



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acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof selected from the following:

- 5 (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6; and
- (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10.

10 Alternatively or in addition, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof and comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, or 37.

15 Alternatively or in addition, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof and comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 7, 9, or 38.

20 As used herein, the term "starch synthase" shall be taken to refer to any enzymatically-active peptide, polypeptide, oligopeptide, polypeptide, protein or enzyme molecule that is at least capable of transferring a glucosyl moiety from ADP-glucose to an  $\alpha$ -1,4-glucan molecule, or a peptide, polypeptide, oligopeptide or polypeptide fragment of such an enzymatically-active molecule.

25

The term "wheat starch synthase" refers to a starch synthase derived from hexaploid wheat or barley or a progenitor species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others, the only requirement that the genomic DNA  
30 is at least about 80% identical to the genome of a wheat plant as determined by standard DNA melting curve analyses.

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The term "starch synthase II" or "wSSII" or similar term shall be taken to refer to a starch synthase as hereinbefore defined that is detectable in the starch granule of a plant seed endosperm and possesses one or more properties selected from the group consisting of:

- 5 (i) it is immunologically cross-reactive with the wheat starch granule proteins designated Sgp-B1 and/or Sgp-D1 and/or Sgp-A1, having estimated molecular weights of about 85 kDa to about 115 kDa;
- (ii) it is encoded by one of a homeologous set of genes localised on wheat chromosomes 7B or 7A or 7D;
- 10 (iii) it is encoded by a nucleotide sequence that comprises at least about 15 nucleotides in length derived from any one or more of SEQ ID NOS: 1, 3, 5, or 37 or a complementary nucleotide sequence thereto;
- (iv) it is encoded by a nucleotide sequence that is at least about 85% identical to one or more of the nucleotide sequences set forth in SEQ ID NOS:
- 15 1, 3, 5, or 37, or a complementary nucleotide sequence thereto;
- (v) it comprises an amino acid sequence having at least about 85% identity to one or more of SEQ ID NOS: 2 or 4 or 6;
- (vi) it comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 20 contiguous amino acids and still even more preferably at least about 25-50 contiguous amino acids of the amino acid sequences set forth in SEQ ID NOS: 2 or 4 or 6;
- 20 (vii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
- 25 (a) KVGGLGDVVTS;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- 30 (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS; and



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(h)TGGLVDTV,

in addition to any one or more of (i) to (vi); and

(viii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- 5 (a) KTGGLGDVAGA;  
(b) GHRVMVVVPRY;  
(c) NDWHTALLPVYLKAYY;  
(d) GIVNGIDNMEWNPEVD;  
(e) DVPLLGFGRDLGQKG;  
10 (f) DVQLVMLGTG;  
(g)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and  
(h)VGG(V/L)RDTV,

in addition to any one or more of (i) to (vi).

15 The term "starch synthase III" or "wSSIII" or similar term shall be taken to refer to a starch synthase as hereinbefore defined that possesses one or more properties selected from the group consisting of:

- (i) it is encoded by a nucleotide sequence that comprises at least about 15 nucleotides in length derived from any one or more of SEQ ID NOS: 7, 9, 11-  
20 16, or 38, or a complementary nucleotide sequence thereto;  
(ii) it is encoded by a nucleotide sequence that is at least about 85% identical to one or more of the nucleotide sequences set forth in SEQ ID NOS: 7, 9, 11-16, or 38, or a complementary nucleotide sequence thereto; and  
(iii) it comprises an amino acid sequence having at least about 85% identity  
25 to one or more of SEQ ID NOS: 8 or 10;  
(iv) it comprises at least about 5 contiguous amino acids, preferably at least about 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 20 contiguous amino acids and still even more preferably at least about 25-50 contiguous amino acids of  
30 the amino acid sequences set forth in SEQ ID NOS: 8 or 10;  
(v) which comprises a conserved amino acid sequence having at least 25%

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identity to an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVVTs;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- 5 (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS; and
- (h) TGGLVDTV

10 in addition to any one or more of (i) to (iv); and

(vi) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KTGGLGDVAGA;
- (b) GHRVMVVVPRY;
- 15 (c) NDWHTALLPVYLKAYY;
- (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFGRDLGQKG;
- (f) DVQLVMLGTG;
- (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- 20 (h) VGG(V/L)RDTV,

in addition to any one or more of (i) to (iv).

In a more preferred embodiment, the WSSII or WSSIII polypeptide encoded by the nucleic acid molecule of the present invention will comprise a substantial contiguous  
25 region of any one of SEQ ID NOS: 2, 4, 6, 8 or 10 or 17 sufficient to possess the biological activity of a starch synthase polypeptide.

For the purposes of nomenclature, the nucleotide sequence set forth in SEQ ID NO: 1 relates to the cDNA molecule encoding the WSSII (i.e. Sgp-B1) polypeptide of  
30 wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:2. The nucleotide sequence set forth in SEQ ID NO: 3 relates to the

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cDNA molecule encoding the WSSII (i.e. Sgp-A1) polypeptide of wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:4. The nucleotide sequence set forth in SEQ ID NO: 5 relates to the cDNA molecule encoding the WSSII (i.e. Sgp-D1) polypeptide of wheat. The amino acid sequence of the corresponding polypeptide is set forth herein as SEQ ID NO:6. The nucleotide sequences set forth in SEQ ID NOS: 7 and 9 relate, respectively, to full-length and partial cDNA molecules encoding the WSSIII polypeptide of wheat. The amino acid sequences of the corresponding polypeptides are set forth herein as SEQ ID NOS: 8 and 10, respectively. The nucleotide sequences set forth in SEQ ID NOS: 11 to 16 relates to fragments of the genomic gene encoding the WSSIII polypeptide of wheat, significant protein-encoding regions of which are described by reference to Table 4 and Figure 11. The nucleotide sequence set forth in SEQ ID NO: 37 relates to the WSSII genomic gene of *Triticum tauschii*, corresponding to the WSSII gene of the D-genome of wheat, which encodes the WSSIII polypeptide. The nucleotide sequence set forth in SEQ ID NO: 38 relates to the wheat WSSIII genomic gene.

Preferably, the isolated nucleic acid molecule of the present invention comprises a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8, or 10 and more preferably, which additionally comprises which comprises one or more amino acid sequences selected from the group consisting of:

- 25 (a) KVGGLGDVVT;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- 30 (f) NGQVLLGSA;
- (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS;

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- (h)TGGLVDTV;  
(i) KTGGLGDVAGA;  
(j) GHRVMVVVPY;  
(k) NDWHTALLPVYLKAYY;  
5 (l) GIVNGIDNMEWNPEVD;  
(m) DVPLLGFGRDLGQKG;  
(n) DVQLVMLGTG;  
(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and  
(p)VGG(V/L)RDTV.

10

The present invention clearly extends to homologues, analogues and derivatives of the wheat starch synthase II and III genes exemplified by the nucleotide sequences set forth herein as SEQ ID NOs: 1, 3, 5, 7, 9, 11-16, 37 or 38.

- 15 Preferred starch synthase genes may be derived from a naturally-occurring starch synthase gene by standard recombinant techniques. Generally, a starch synthase gene may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the starch synthase gene of the present invention include 5' and 3' terminal fusions as  
20 well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the  
25 sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or  
30 hydrophobicity.

For the present purpose, "homologues" of a nucleotide sequence shall be taken to

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refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

5

"Analogues" of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence of any non-nucleotide constituents not normally  
10 present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a nucleotide sequence set forth herein shall be taken to refer to any  
15 isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5' and 3' terminal fusions as well  
20 as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are  
25 characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

30 The present invention extends to the isolated nucleic acid molecule when integrated into the genome of a cell as an addition to the endogenous cellular complement of starch synthase genes, irrespective of whether or not the introduced nucleotide



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sequence is translatable or non-translatable to produce a polypeptide. The present invention clearly contemplates the introduction of additional copies of starch synthase genes into plants, particularly wheat plants, in the antisense orientation to reduce the expression of particular wheat starch synthase genes. As will be known to those skilled  
5 in the art, such antisense genes are non-translatable, notwithstanding that they can be expressed to produce antisense mRNA molecules.

The said integrated nucleic acid molecule may, or may not, contain promoter sequences to regulate expression of the subject genetic sequence.

10

Accordingly, the present invention clearly encompasses preferred homologues, analogues and derivatives that comprise a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof  
15 selected from the following:

- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- 20 (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;
- (iii) a wheat starch synthase polypeptide, protein or enzyme or functional  
25 subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
  - (a) KVGGLGDVWTS;
  - (b) GHTVEVILPKY;
  - 30 (c) HDWSSAPVAWLYKEHY;
  - (d) GILNGIDPDIWDPYTD;

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- (e) DVPIVGIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS; and
- (h) TGGLVDTV

5 and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at

10 least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KTGGLGDVAGA;
- (b) GHRVMVVVPRY;
- (c) NDWHTALLPVYLKAYY;
- 15 (d) GIVNGIDNMEWNPEVD;
- (e) DVPLLGFGRDLGQKG;
- (f) DVQLVMLGTG;
- (g) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (h) VGG(V/L)RDTV,

20 and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10.

Preferably, the isolated nucleic acid molecule encodes a starch synthase polypeptide,

25 protein or enzyme that comprises two, more preferably three, more preferably four, more preferably five, more preferably six, more preferably seven and even more preferably eight of the conserved amino acid motifs listed *supra*. Even more preferably, the said amino acid motifs are located in a relative configuration such as that shown for the wheat SSII or wheat SSIII polypeptides described herein.

30

In a preferred embodiment, the isolated nucleic acid molecule encodes a starch



- 30 -

synthase polypeptide, protein or enzyme having at least about 90% amino acid sequence identity to any one of SEQ ID NOS: 2, 4, 6, 8 or 10, more preferably having at least about 95% or about 97% or about 99% identity to any one of said amino acid sequences.

5

In an alternative embodiment, the present invention provides an isolated nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof, wherein said nucleic acid molecule comprises a nucleotide sequence having at least about 85% nucleotide sequence identity to any  
10 one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a degenerate nucleotide sequence thereto or a complementary nucleotide sequence thereto.

By "degenerate nucleotide sequence" is meant a nucleotide sequence that encodes a substantially identical amino acid sequence as a stated nucleotide sequence.

15

In a preferred embodiment, the isolated nucleic acid molecule comprises the nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or is at least about 90% identical, more preferably at least about 95% or 97% or 99% identical to all or a protein-encoding part thereof.

20

In an alternative embodiment, preferred homologues, analogues and derivatives of the nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof and comprises a nucleotide sequence that is capable of hybridising under at least moderate  
25 stringency hybridisation conditions to at least about 30 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto.

For the purposes of defining the level of stringency, a low stringency is defined herein  
30 as being a hybridisation and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C. Generally, the stringency is increased by reducing the concentration of SSC

buffer, and/or increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. A moderate stringency comprises a hybridisation and/or a wash carried out in 0.2 x SSC-2 x SSC buffer, 0.1% (w/v) SDS at 42°C to 65°C, while a high stringency comprises a hybridisation and/or a wash carried out in 5 0.1xSSC-0.2 x SSC buffer, 0.1% (w/v) SDS at a temperature of at least 55°C. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of further clarification only, reference to the parameters affecting hybridisation between nucleic acid molecules is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

10

Those skilled in the art will be aware of procedures for the isolation of further wheat starch synthase genes to those specifically described herein or homologues, analogues or derivatives of said genes, for example further cDNA sequences and genomic gene equivalents, when provided with one or more of the nucleotide 15 sequences set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11-16, 37, or 38. In particular, amplifications and/or hybridisations may be performed using one or more nucleic acid primers or hybridisation probes comprising at least 10 contiguous nucleotides and preferably at least about 20 contiguous nucleotides or 50 contiguous nucleotides derived from the nucleotide sequences set forth herein, to isolate cDNA clones, mRNA 20 molecules, genomic clones from a genomic library (in particular genomic clones containing the entire 5' upstream region of the gene including the promoter sequence, and the entire coding region and 3'-untranslated sequences), and/or synthetic oligonucleotide molecules, amongst others. The present invention clearly extends to such related sequences.

25

Accordingly, a second aspect of the present invention provides a method of isolating a nucleic acid molecule that encodes a starch synthase polypeptide, protein or enzyme said method comprising:

- 30 (i) hybridising a probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto to single-stranded or double-stranded mRNA, cDNA or genomic DNA; and

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- (ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.

Preferably, the detecting means is a reporter molecule covalently attached to the probe  
5 or primer molecule or alternatively, a polymerase chain reaction format.

An alternative method contemplated in the present invention involves hybridising two nucleic acid "primer molecules" to a nucleic acid "template molecule" which comprises a related starch synthase gene or related starch synthase genetic sequence or a  
10 functional part thereof, wherein the first of said primers comprises contiguous nucleotides derived from any one or more of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, and the second of said primers comprises contiguous nucleotides complementary to any one or more of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38. Specific nucleic acid molecule copies of the template molecule are amplified enzymatically in a  
15 polymerase chain reaction, a technique that is well known to one skilled in the art.

In a preferred embodiment, each nucleic acid primer molecule is at least 10 nucleotides in length, more preferably at least 20 nucleotides in length, even more preferably at least 30 nucleotides in length, still more preferably at least 40 nucleotides  
20 in length and even still more preferably at least 50 nucleotides in length.

Furthermore, the nucleic acid primer molecules consists of a combination of any of the nucleotides adenine, cytidine, guanine, thymidine, or inosine, or functional analogues or derivatives thereof which are at least capable of being incorporated into a  
25 polynucleotide molecule without having an inhibitory effect on the hybridisation of said primer to the template molecule in the environment in which it is used.

Furthermore, one or both of the nucleic acid primer molecules may be contained in an aqueous mixture of other nucleic acid primer molecules, for example a mixture of  
30 degenerate primer sequences which vary from each other by one or more nucleotide substitutions or deletions. Alternatively, one or both of the nucleic acid primer molecules may be in a substantially pure form.

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The nucleic acid template molecule may be in a recombinant form, in a virus particle, bacteriophage particle, yeast cell, animal cell, or a plant cell. Preferably, the nucleic acid template molecule is derived from a plant cell, tissue or organ, in particular a cell, tissue or organ derived from a wheat or barley plant or a progenitor species, or a  
5 relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others.

Those skilled in the art will be aware that there are many known variations of the basic polymerase chain reaction procedure, which may be employed to isolate a related  
10 starch synthase gene or related starch synthase genetic sequence when provided with the nucleotide sequences set forth herein. Such variations are discussed, for example, in McPherson *et al* (1991). The present invention extends to the use of all such variations in the isolation of related starch synthase genes or related starch synthase genetic sequences using the nucleotide sequences embodied by the present invention.

15

As exemplified herein, the present inventors have isolated several wheat starch synthase genes using both hybridisation and polymerase chain reaction approaches, employing novel probes and primer sequences to do so.

20 Accordingly, a third aspect of the invention provides an isolated probe or primer comprising at least about 15 contiguous nucleotides in length derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto.

25 Preferably, the probe or primer comprises a nucleotide sequence set forth in any one of SEQ ID NOS: 25 to 34.

The isolated nucleic acid molecule of the present invention may be introduced into and expressed in any cell, for example a plant cell, fungal cell, insect cell, animal cell, yeast  
30 cell or bacterial cell. Those skilled in the art will be aware of any modifications which are required to the codon usage or promoter sequences or other regulatory

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sequences, in order for expression to occur in such cells.

A further aspect of the invention provides a method of assaying for the presence or absence of a starch synthase isoenzyme or the copy number of a gene encoding same  
5 in a plant, comprising contacting a biological sample derived from said plant with an isolated nucleic acid molecule derived from any one of SEQ ID NOS 1, 3, 5, 7, 9, 11-16, 37, or 38, or any one of SEQ ID NOS: 25 to 34, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

10

The detection means according to this aspect of the invention is any nucleic acid based hybridisation or amplification reaction.

The hexaploid nature of wheat prevents the straightforward identification of starch  
15 synthase allelic variants by hybridisation using the complete starch synthase-encoding sequence, because the similarities between the various alleles generally results in significant cross-hybridisation. Accordingly, sequence-specific hybridisation probes are required to distinguish between the various alleles. Similarly, wherein PCR is used to amplify specific allelic variants of a starch synthase gene, one or more sequence-  
20 specific amplification primers are generally required. As will be apparent from the amino acid sequence comparisons provided herein, such as in Figures 3 and 13, non-conserved regions of particular wheat starch synthase polypeptides are particularly useful for the design of probes and primers that are capable of distinguishing between one or more starch synthase polypeptide isoenzyme or allelic variant. The present  
25 invention clearly contemplates the design of such probes and primers based upon the sequence comparisons provided herein.

In the performance of this embodiment of the present invention, the present inventors particularly contemplate the identification of wheat starch synthase null alleles or  
30 alternatively, mutations wherein specific amino acids are inserted or deleted or substituted, compared to one or more of the wheat SSII or SSIII alleles disclosed



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herein. Such null alleles and other allelic variants are readily identifiable using PCR screening which employs amplification primers based upon the nucleotide and amino acid sequences disclosed herein for SSII and/or SSIII. Once identified, the various mutations can be stacked or pyramided into one or more new wheat lines, such as by  
5 introgression and/or standard plant breeding and/or recombinant approaches (eg. transformation, transfection, etc) thereby producing a novel germplasm which exhibits altered starch properties compared to existing lines. DNA markers based upon the nucleotide and amino acid sequences disclosed herein for SSII and/or SSIII can be employed to monitor the stacking of genes into the new lines and to correlate the  
10 presence of particular genes with starch phenotypes of said lines.

In this regard, a significant advantage conferred by the present invention is the design of new DNA markers that reveal polymorphisms such as, for example, length polymorphisms, restriction site polymorphisms, and single nucleotide polymorphisms,  
15 amongst others, between wheat starch synthases and, in particular, between wheat GBSS and/or SSI and/or SSII and/or SSIII, or between allelic variants of one or more of said starch synthases, that can be used to identify the three genomes of hexaploid wheats (i.e., the A, B and D genomes).

20 Preferably, such DNA markers are derived from the intron region of a starch synthase gene disclosed herein, more preferably the wheat SSII and/or the wheat SSIII gene. Those skilled in the art will be aware that such regions generally have a higher degree of variation than in the protein-encoding regions and, as a consequence, are particularly useful in identifying specific allelic variants of a particular gene, such as  
25 allelic variants contained in any one of the three wheat genomes, or alternatively or in addition, for the purpose of distinguishing between wheat GBSS, SSI, SSII or SSIII genes.

A further approach contemplated by the present inventors is the design of unique  
30 isoenzyme-specific and/or allele-specific peptides based upon the amino acid sequence disclosed herein as SEQ ID NOS: 25 and/or SEQ ID NO: 4 and/or SEQ ID

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NO: 6 and/or SEQ ID NO: 8 and/or SEQ ID NO: 10, which peptides are then used to produce polyclonal or monoclonal antibodies by conventional means. Alternatively, the genes encoding these polypeptides or unique peptide regions thereof can be introduced in an expressible format into an appropriate prokaryotic or eukaryotic  
5 expression system, where they can be expressed to produce the isoenzyme-specific and/or allele-specific peptides for antibody production. Such antibodies may also be used as markers for the purpose of both identifying parental lines and germplasms and monitoring the stacking of genes in new lines, using conventional immunoassays such as, for example, ELISA and western blotting.

10

A further aspect of the present invention utilises the above-mentioned nucleic acid based assay method in the breeding and/or selection of plants which express or do not express particular starch synthase isoenzymes or alternatively, which express a particular starch synthase isoenzyme at a particular level in one or more plant tissues.  
15 This aspect clearly extends to the selection of transformed plant material which contains one or more of the isolated nucleic acid molecules of the present invention.

Yet another aspect of the present invention provides for the expression of the nucleic acid molecule of the present invention in a suitable host (e.g. a prokaryote or  
20 eukaryote) to produce full length or non-full length recombinant starch synthase gene products.

Hereinafter the term "starch synthase gene product" shall be taken to refer to a recombinant product of a starch synthase gene of the present invention.

25

Preferably, the recombinant starch synthase gene product comprises an amino acid sequence having the catalytic activity of a starch synthase polypeptide or a functional mutant, derivative part, fragment, or analogue thereof.

30 In a particularly preferred embodiment of the invention, the recombinant starch synthase gene product is selected from the following:



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- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- 5 (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10; and
- 10 (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:
- (a) KVGGLGDVVT;
  - (b) GHTVEVILPKY;
  - 15 (c) HDWSSAPVAWLYKEHY;
  - (d) GILNGIDPDIWDPYTD;
  - (e) DVPIVGIITRLTAQKG;
  - (f) NGQVVLLGSA;
  - (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS;
  - 20 (h) TGGLVDTV;
- (i) a wheat starch synthase II (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, or 6;
- 25 (ii) a wheat starch synthase III (wSSIII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 8 or 10;
- 30 (iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at

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least 25% identity to an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVVT;
- (b) GHTVEVILPKY;
- 5 (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- (f) NGQVVLLGSA;
- (g) AGSDFIIVPSIFEPCGLTQLVAMRYGS; and
- 10 (h) TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPRY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- 15 (m) DVPLLGFGRDLGQKG;
- (n) DVQLVMLGTG;
- (o) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (p) VGG(V/L)RDTV.

20 Accordingly, the present invention clearly extends to homologues, analogues and derivatives of the amino acid sequences set forth herein as SEQ ID NOS: 2, 4, 6, 8 and 10.

In the present context, "homologues" of an amino acid sequence refer to those  
25 polypeptides, enzymes or proteins which have a similar catalytic activity to the amino acid sequences described herein, notwithstanding any amino acid substitutions, additions or deletions thereto. A homologue may be isolated or derived from the same or another plant species as the species from which the polypeptides of the invention are derived.

30

"Analogues" encompass polypeptides of the invention notwithstanding the occurrence

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of any non-naturally occurring amino acid analogues therein.

"Derivatives" include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, derivatives of an amino acid sequence described herein which comprises fragments or parts of the subject amino acid sequences are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more copies of the subject polypeptides. Procedures for derivatizing peptides are well-known in the art.

Substitutions encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which an amino acid residue contained in a starch synthase gene product is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a starch synthase gene product described herein is substituted with an amino acid with different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

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Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions  
5 and of the order of 1-4 amino acid residues.

A homologue, analogue or derivative of a starch synthase gene product as referred to herein may readily be made using peptide synthetic techniques well-known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA  
10 manipulations. Techniques for making substituent mutations at pre-determined sites using recombinant DNA technology, for example by M13 mutagenesis, are also well-known. The manipulation of nucleic acid molecules to produce variant peptides, polypeptides or proteins which manifest as substitutions, insertions or deletions are well-known in the art.

15

The starch synthase gene products described herein may be derivatized further by the inclusion or attachment thereto of a protective group which prevents, inhibits or slows proteolytic or cellular degradative processes. Such derivatization may be useful where the half-life of the subject polypeptide is required to be extended, for example to  
20 increase the amount of starch produced in the endosperm or alternatively, to increase the amount of protein produced in a bacterial or eukaryotic expression system. Examples of chemical groups suitable for this purpose include, but are not limited to, any of the non-conventional amino acid residues listed in Table 2, in particular a D-stereoisomer or a methylated form of a naturally-occurring amino acid listed in Table  
25 1. Additional chemical groups which are useful for this purpose are selected from the list comprising aryl or heterocyclic N-acyl substituents, polyalkylene oxide moieties, desulphatohirudin muteins, alpha-muteins, alpha-aminophosphonic acids, water-soluble polymer groups such as polyethylene glycol attached to sugar residues using hydrazone or oxime groups, benzodiazepine dione derivatives, glycosyl groups such  
30 as beta-glycosylamine or a derivative thereof, isocyanate conjugated to a polyol functional group or polyoxyethylene polyol capped with diisocyanate, amongst others. Similarly, a starch synthase gene product or a homologue, analogue or derivative

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thereof may be cross-linked or fused to itself or to a protease inhibitor peptide, to reduce susceptibility of said molecule to proteolysis.

In a particularly preferred embodiment, the percentage similarity to in any one of SEQ  
5 ID NOS: 2, 4, 6, 8 or 10 is at least about 90%, more preferably at least about 95%,  
even more preferably at least about 97% and even more preferably at least about  
98%, or about 99% or 100%.

In a related embodiment, the present invention provides a "sequencably pure" form of  
10 the amino acid sequence described herein. "Sequencably pure" is hereinbefore  
described as substantially homogeneous to facilitate amino acid determination.

In a further related embodiment, the present invention provides a "substantially  
homogeneous" form of the subject amino acid sequence, wherein the term  
15 "substantially homogeneous" is hereinbefore defined as being in a form suitable for  
interaction with an immunologically interactive molecule. Preferably, the polypeptide  
is at least 20% homogeneous, more preferably at least 50% homogeneous, still more  
preferably at least 75% homogeneous and yet still more preferably at least about 95-  
100% homogenous, in terms of activity per microgram of total protein in the protein  
20 preparation.

To produce the recombinant polypeptide of the present invention, the coding region  
of a starch synthase gene described herein or a functional homologue, analogue or  
derivative thereof is placed operably in connection with a promoter sequence in the  
25 sense orientation, such that a starch synthase gene product is capable of being  
expressed under the control of said promoter sequence.

In the present context, the term "in operable connection with" means that expression  
of the isolated nucleotide sequence is under the control of the promoter sequence with  
30 which it is connected, regardless of the relative physical distance of the sequences  
from each other or their relative orientation with respect to each other.



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Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. A promoter is usually, but not necessarily, positioned upstream or 5', of a structural gene, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

10

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a structural gene or other nucleic acid molecule, particularly in a plant cell and more preferably in a wheat plant or other monocotyledonous plant cell, tissue or organ. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression and/or to alter the spatial expression and/or temporal expression. For example, regulatory elements which confer copper inducibility may be placed adjacent to a heterologous promoter sequence, thereby conferring copper inducibility on the expression of said molecule.

20

Those skilled in the art will be aware that in order to obtain optimum expression of the starch synthase gene of the present invention, it is necessary to position said gene in an appropriate configuration such that expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from

30



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which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for expressing the starch synthase gene of the present invention include viral, fungal, bacterial, animal and plant derived promoters capable of functioning in prokaryotic or eukaryotic cells. Preferred promoters are those capable of regulating the expression of the subject starch synthase genes in plants cells, fungal cells, insect cells, yeast cells, animal cells or bacterial cells, amongst others. Particularly preferred promoters are capable of regulating expression of the subject nucleic acid molecules in monocotyledonous plant cells. The promoter may regulate the expression of the said molecule constitutively, or differentially with respect to the tissue in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or plant pathogens, or metal ions, amongst others.

15

Accordingly, strong constitutive promoters are particularly preferred for the purposes of the present invention.

Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* operator-promoter, *tac* promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, CaMV 35S promoter, SCSV promoter, SCBV promoter and the like.

Particularly preferred promoters operable in plant cells include, for example the CaMV 35S promoter, and the SCBV promoter. Those skilled in the art will readily be aware of additional promoter sequences other than those specifically described.

In a particularly preferred embodiment, the promoter may be derived from a genomic starch synthase gene. Preferably, the promoter sequence comprises nucleotide sequences that are linked *in vivo* to nucleotide sequences set forth in any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11-16, 37, or 38. By "linked *in vivo*" means that the promoter is present in its native state in the genome of a wheat plant where it controls expression

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of the starch synthase gene of the present invention.

Conveniently, genetic constructs are employed to facilitate expression of a starch synthase genetic sequence of the present invention or a functional derivative, part,  
5 homologue, or analogue thereof. To produce a genetic construct, the starch synthase gene of the invention is inserted into a suitable vector or episome molecule, such as a bacteriophage vector, viral vector or a plasmid, cosmid or artificial chromosome vector which is capable of being maintained and/or replicated and/or expressed in the host cell, tissue or organ into which it is subsequently introduced. The said genetic  
10 construct comprises the subject nucleic acid molecule placed operably under the control of a promoter sequence and optionally, a terminator sequence.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA  
15 sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in bacteria, yeasts, animal cells and plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

20 Examples of terminators particularly suitable for use in expressing the nucleic acid molecule of the present invention in plant cells include the nopaline synthase (NOS) gene terminator of *Agrobacterium tumefaciens*, the terminator of the Cauliflower mosaic virus (CaMV) 35S gene, and the *zein* gene terminator from *Zea mays*.

25 Genetic constructs will generally further comprise one or more origins of replication and/or selectable marker gene sequences.

The origin of replication can be functional in a bacterial cell and comprise, for example, the pUC or the ColE1 origin. Alternatively, the origin of replication is operable in a  
30 eukaryotic cell, tissue and more preferably comprises the 2 micron (2 $\mu$ m) origin of replication or the SV40 origin of replication.

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As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a genetic construct of the invention or a derivative thereof.

5

Suitable selectable marker genes contemplated herein include the ampicillin-resistance gene (Amp<sup>r</sup>), tetracycline-resistance gene (Tc<sup>r</sup>), bacterial kanamycin-resistance gene (Kan<sup>r</sup>), is the zeocin resistance gene (Zeocin is a drug of bleomycin family which is trademark of InVitrogen Corporation), the *AURI-C* gene which confers resistance to the  
10 antibiotic aureobasidin A, phosphinothricin-resistance gene, neomycin phosphotransferase gene (*nptII*), hygromycin-resistance gene,  $\beta$ -glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein-encoding gene or the luciferase gene, amongst others. Those skilled in the art will be aware of other selectable marker genes useful in the performance of the present  
15 invention and the subject invention is not limited by the nature of the selectable marker gene.

Usually, an origin of replication or a selectable marker gene suitable for use in bacteria is physically-separated from those genetic sequences contained in the genetic  
20 construct which are intended to be expressed or transferred to a eukaryotic cell, or integrated into the genome of a eukaryotic cell.

Standard methods can be used to introduce genetic constructs into a cell, tissue or organ for the purposes of modulating gene expression. Particularly preferred methods  
25 suited to the introduction of synthetic genes and genetic constructs comprising same to eukaryotic cells include liposome-mediated transfection or transformation, transformation of cells with attenuated virus particles or bacterial cells and standard procedures for the transformation of plant and animal cells, tissues, organs or organisms. Any standard means may be used for their introduction including cell  
30 mating, transformation or transfection procedures known to those skilled in the art or described by Ausubel *et al.* (1992).

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In a further embodiment of the present invention, the starch synthase genes of the present invention and genetic constructs comprising same are adapted for integration into the genome of a cell in which it is expressed. Those skilled in the art will be aware that, in order to achieve integration of a genetic sequence or genetic construct into the  
5 genome of a host cell, certain additional genetic sequences may be required. In the case of plants, left and right border sequences from the T-DNA of the *Agrobacterium tumefaciens* Ti plasmid will generally be required.

The invention further contemplates increased starch and/or modified starch  
10 composition in transgenic plants expressing the nucleic acid molecule of the invention in the sense orientation such that the activity of one or more starch synthase isoenzymes is increased therein. By increasing the level of one or more starch synthase isoenzymes, the deposition of starch in the amyloplast or chloroplast is increased and/or a modified starch granule structure is produced and/or starch  
15 composition is modified and/or the amylose/amylopectin ratio is altered in the plant.

Wherein it is desired to increase the synthesis of a particular starch synthase isoenzyme in a plant cell, the coding region of a starch synthase gene is placed operably behind a promoter, in the sense orientation, such that said starch synthase  
20 is expressed under the control of said promoter sequence. In a preferred embodiment, the starch synthase genetic sequence is a starch synthase genomic sequence, cDNA molecule or protein-coding sequence.

Wherein it is desirable to reduce the level of a particular starch synthase isoenzyme  
25 in a plant cell, the nucleic acid molecule of the present invention can be expressed in the antisense orientation, as an antisense molecule or a ribozyme molecule, under the control of a suitable promoter.

Alternatively, the nucleic acid molecule of the present invention may also be expressed  
30 in the sense orientation, in the form of a co-suppression molecule, to reduce the level of a particular starch synthase isoenzyme in a plant cell. As will be known to those skilled in the art, co-suppression molecules that comprise inverted repeat sequences

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of a target nucleic acid molecule provide optimum efficiency at reducing expression of said target nucleic acid molecule and, as a consequence, the present invention clearly contemplates the use of inverted repeat sequences of any one or more of the starch synthase genetic sequences exemplified herein, or inverted repeat sequences of a  
5 homologue, analogue or derivative of said starch synthase genetic sequences, to reduce the level of a starch synthase isoenzyme in a plant.

The expression of an antisense, ribozyme or co-suppression molecule comprising a starch synthase gene in a cell such as a plant cell, fungal cell, insect cell, animal cell,  
10 yeast cell or bacterial cell, may also increase the availability of carbon as a precursor for a secondary metabolite other than starch (e.g. sucrose or cellulose). By targeting the endogenous starch synthase gene, expression is diminished, reduced or otherwise lowered to a level that results in reduced deposition of starch in the amyloplast or chloroplast and/or leads to modified starch granule structure and/or composition  
15 and/or altered amylose/amylopectin ratio.

Accordingly, a further aspect of the present invention provides a method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing therein a sense molecule, antisense molecule, ribozyme  
20 molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified. This aspect of the invention clearly extends to the  
25 introduction of the sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule to isolated plant cells, tissues or organs or organelles by cell fusion or transgenic means and the regeneration of intact plants therefrom.

30 Co-suppression is the reduction in expression of an endogenous gene that occurs when one or more copies of said gene, or one or more copies of a substantially similar



gene are introduced into the cell, preferably in the form of an inverted repeat structure.

The present inventors have discovered that the genetic sequences disclosed herein are capable of being used to modify the level of starch when expressed, particularly  
5 when expressed in plants cells. Accordingly, the present invention clearly extends to the modification of starch biosynthesis in plants, in particular wheat or barley plants or a progenitor plant species, or a relative thereto such as the diploid *Triticum tauschii* or other diploid, tetraploid, aneuploid, polyploid, nullisomic, or a wheat/barley addition line, amongst others.

10

In particular, the present invention contemplates decreased starch production and/or modified starch composition in transgenic plants expressing the nucleic acid molecule of the invention in the antisense orientation or alternatively, expressing a ribozyme or co-suppression molecule comprising the nucleic acid sequence of the invention such  
15 that the activity of one or more starch synthase isoenzymes is decreased therein.

In the context of the present invention, an antisense molecule is an RNA molecule  
20 which is transcribed from the complementary strand of a nuclear gene to that which is normally transcribed to produce a "sense" mRNA molecule capable of being translated into a starch synthase polypeptide. The antisense molecule is therefore complementary to the mRNA transcribed from a sense starch synthase gene or a part thereof. Although not limiting the mode of action of the antisense molecules of the  
25 present invention to any specific mechanism, the antisense RNA molecule possesses the capacity to form a double-stranded mRNA by base pairing with the sense mRNA, which may prevent translation of the sense mRNA and subsequent synthesis of a polypeptide gene product.

30 Ribozymes are synthetic RNA molecules which comprise a hybridising region complementary to two regions, each of at least 5 contiguous nucleotide bases in the target sense mRNA. In addition, ribozymes possess highly specific endoribonuclease



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activity, which autocatalytically cleaves the target sense mRNA. A complete description of the function of ribozymes is presented by Haseloff and Gerlach (1988) and contained in International Patent Application No. WO89/05852.

- 5 The present invention extends to ribozyme which target a sense mRNA encoding a native starch synthase gene product, thereby hybridising to said sense mRNA and cleaving it, such that it is no longer capable of being translated to synthesise a functional polypeptide product.
- 10 According to this embodiment, the present invention provides a ribozyme or antisense molecule comprising at least 5 contiguous nucleotide bases derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto or a homologue, analogue or derivative thereof, wherein said antisense or ribozyme molecule is able to form a hydrogen-bonded complex with a sense mRNA
- 15 encoding a starch synthase gene product to reduce translation thereof.

In a preferred embodiment, the antisense or ribozyme molecule comprises at least 10 to 20 contiguous nucleotides derived from any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto or a homologue, analogue

20 or derivative thereof. Although the preferred antisense and/or ribozyme molecules hybridise to at least about 10 to 20 nucleotides of the target molecule, the present invention extends to molecules capable of hybridising to at least about 50-100 nucleotide bases in length, or a molecule capable of hybridising to a full-length or substantially full-length mRNA encoded by a starch synthase gene.

25

Those skilled in the art will be aware of the necessary conditions, if any, for selecting or preparing the antisense or ribozyme molecules of the invention.

It is understood in the art that certain modifications, including nucleotide substitutions

30 amongst others, may be made to the antisense and/or ribozyme molecules of the present invention, without destroying the efficacy of said molecules in inhibiting the expression of a starch synthase gene. It is therefore within the scope of the present

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invention to include any nucleotide sequence variants, homologues, analogues, or fragments of the said gene encoding same, the only requirement being that said nucleotide sequence variant, when transcribed, produces an antisense and/or ribozyme molecule which is capable of hybridising to a sense mRNA molecule which  
5 encodes a starch synthase gene product.

Gene targeting is the replacement of an endogenous gene sequence within a cell by a related DNA sequence to which it hybridises, thereby altering the form and/or function of the endogenous gene and the subsequent phenotype of the cell. According  
10 to this embodiment, at least a part of the DNA sequence defined by any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38 may be introduced into target cells containing an endogenous gene that encodes a particular starch synthase isoenzyme, thereby replacing said endogenous gene. According to this embodiment, the polypeptide product of the gene targeting molecule generally encodes a starch synthase  
15 isoenzyme that possesses different catalytic activity to the polypeptide product of the endogenous gene, producing in turn modified starch content and/or composition in the target cell.

The present invention extends to genetic constructs designed to facilitate expression  
20 of a sense molecule, an antisense molecule, ribozyme molecule, co-suppression molecule, or gene targeting molecule of the present invention. The requirements for expressing such molecules are similar to those for expressing a recombinant polypeptide as described *supra*.

25 The present invention further extends to the production and use of starches and proteins produced using the novel genes described herein. Modified starches produced by plants which have been selected using marker-assisted selection, or alternatively, produced by transgenic plants carrying the introduced starch synthase genes, are particularly suitable for use in food products, such as, for example, flour  
30 and flour-based products, in particular those products selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry. Modified proteins are also suitable for use in non-

food products, such as, for example, those non-food products selected from the group consisting of: films; coatings; adhesives; building materials; and packaging materials.

Additionally, starch hydrolysates or undegraded starches are both useful in industry  
5 and, as a consequence, the present invention is useful in applications relating to the use of both starch hydrolysates and undegraded starches. By "starch hydrolysates" is meant the glucose and glucan components that are obtainable by the enzymatic or chemical degradation of starch in chemical modifications and processes, such as fermentation.

10

Starch produced by plants expressing the sense, antisense, co-suppression, gene-targeting or ribozyme molecules of the present invention may exhibit modified viscosities and/or gelling properties of its glues when compared to starch derived from wild-type plants. Native starches produced by the performance of the inventive method  
15 are useful as an additive in the following: (i) foodstuffs, for the purpose of increasing the viscosity or gelling properties of food; (ii) in non-foodstuffs, such as an adjuvant or additive in the paper and cardboard industries, for retention or as a size filler, or as a solidifying substance or for dehydration, or film coating, amongst others; (iii) in the adhesive industry as pure starch glue, as an additive to synthetic resins and polymer  
20 dispersions, or as an extenders for synthetic adhesives; (iv) in the textile and textile care industries to strengthen woven products and reduce burring or to thicken dye pastes; (v) in the building industry, such as a binding agent in the production of gypsum plaster boards, or for the deceleration of the sizing process; (vi) in ground stabilization or for the temporary protection of ground particles against water in artificial  
25 earth shifting; (vii) as a wetting agent in plant protectants and fertilizers; (viii) as a binding agent in drugs, pharmaceuticals and medicated foodstuff such as vitamins, etc; (ix) as an additive in coal and briquettes; (xi) as a flocculent in the processing of coal ore and slurries; (xii) as a binding agent in casting processes to increase flow resistance and improve binding strength; and (xiii) to improve the technical and optical  
30 quality of rubber and plastic products. Additional applications are not excluded.

A further aspect of the present invention provides an isolated promoter that is operable

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in the endosperm of a monocotyledonous plant cell, tissue or organ, and preferably in the endosperm of a monocotyledonous plant cell, tissue or organ. According to this embodiment, it is preferred that the promoter is derived from a starch synthase gene of the present invention, such as a promoter that is linked *in vivo* to any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto.

In a particularly preferred embodiment, the promoter comprises a nucleotide sequence derivable from the 5'-upstream region of SEQ ID NO: 11 or SEQ ID NO: 37 or SEQ ID NO: 38, or a complementary nucleotide sequence thereto, and more preferably comprises nucleotides 1 to about 287 of SEQ ID NO: 11, or nucleotides 1 to about 1416 of SEQ ID NO: 37, or nucleotides 1 to about 973 of SEQ ID NO: 38, or a complementary nucleotide sequence thereto. The present invention clearly extends to promoter sequences that comprise further nucleotide sequences in the region upstream of the stated nucleotide sequence that are linked *in vivo* to said nucleotide sequence in the wheat genome.

In a related embodiment, the promoter sequence of the present invention will further comprise an exon sequence derived from a starch synthase gene, such as, for example, an intron I sequence described herein, or a complementary nucleotide sequence thereto. Those skilled in the art will be aware that the inclusion of such nucleotide sequences may increase the expression of a heterologous structural gene, the expression of which is controlled thereby. Preferred intron I sequences include, for example, nucleotide sequences in the region of about position 1744 to about 1847 of SEQ ID NO: 37, and/or about position 1100 to about position 2056 of SEQ ID NO: 38. Additional sequences comprising intron/exon junction boundary sequences which are readily determined by those skilled in the art are not excluded.

The present invention further extends to the expression of any structural gene operably under the control of the starch synthase promoter sequence exemplified herein or a functional homologue, analogue or derivative of said promoter sequence.

As with other embodiments described herein for expression in cells, a genetic construct may be employed to effect said expression and the present invention clearly extends to said genetic constructs.

- 5 The polypeptide encoded by the structural gene component may be a reporter molecule which is encoded by a gene such as the bacterial  $\beta$ -glucuronidase gene or chloramphenicol acetyltransferase gene or alternatively, the firefly luciferase gene. Alternatively, wherein it is desirable to alter carbon partitioning within the endosperm, the polypeptide may be an enzyme of the starch sucrose biosynthetic pathways.
- 10 Preferably, the promoter sequence is used to regulate the expression of one or more of the starch synthase genes of the present invention or a sense, antisense, ribozyme, co-suppression or gene-targetting molecule comprising or derived from same.

Recombinant DNA molecules carrying the aforesaid nucleic acid molecule of the

15 present invention or a sense, antisense, ribozyme, gene-targetting or co-suppression molecule and/or genetic construct comprising same, may be introduced into plant tissue, thereby producing a "transgenic plant", by various techniques known to those skilled in the art. The technique used for a given plant species or specific type of plant tissue depends on the known successful techniques. Means for introducing

20 recombinant DNA into plant tissue include, but are not limited to, transformation (Paszowski *et al.*, 1984), electroporation (Fromm *et al.*, 1985), or microinjection of the DNA (Crossway *et al.*, 1986), or T-DNA-mediated transfer from *Agrobacterium* to the plant tissue. Representative T-DNA vector systems are described in the following references: An *et al.* (1985); Herrera-Estrella *et al.* (1983a, b); Herrera-Estrella *et al.*

25 (1985). Once introduced into the plant tissue, the expression of the introduced gene may be assayed in a transient expression system, or it may be determined after selection for stable integration within the plant genome. Techniques are known for the *in vitro* culture of plant tissue, and in a number of cases, for regeneration into whole plants. Procedures for transferring the introduced gene from the originally transformed

30 plant into commercially useful cultivars are known to those skilled in the art.

In general, plants are regenerated from transformed plant cells or tissues or organs on



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hormone-containing media and the regenerated plants may take a variety of forms, such as chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). Transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plants may be selfed to give homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques.

10

Accordingly, a still further aspect of the present invention contemplates a transgenic plant comprising an introduced sense molecule, antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule having at least about 85% nucleotide sequence identity to any one of any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11-16, 37, or 38, or a complementary nucleotide sequence thereto or a genetic construct comprising same. The present invention further extends to those plant parts, propagules and progeny of said transgenic plant or derived therefrom, the only requirement being that said propagules and progeny also carry the introduced nucleic acid molecule(s).

20

The present invention is further described by reference to the following non-limiting examples.

### EXAMPLE 1

#### Plant material

Genetic stocks of hexaploid bread wheat *Triticum aestivum* cv. Chinese Spring with various chromosome additions and deletions were kindly supplied by Dr E. Lagudah (CSIRO Plant Industry, Canberra) and derived from stocks described in Sears and Miller (1985). The hexaploid (*Triticum aestivum*) wheats cv Gabo and cv Wyuna were grown in controlled growth cabinet conditions (18°C day and 13°C night, with a photoperiod of 16 h). Wheat leaves and florets prior to anthesis, and endosperm were collected over the grain filling period, immediately frozen in liquid nitrogen and stored

25  
30



at -80°C until required.

## EXAMPLE 2

### Gel Electrophoresis, Antibodies and Immunoblotting

5 Monoclonal antibodies against the Sgp-1 proteins, and their use in the immunoblotting of SDS-PAGE gels have been described previously (Rahman *et al.*, 1995).

## EXAMPLE 3

### Preparation of total RNA from wheat

10 Total RNA was isolated from the leaf, floret and endosperm tissues of wheat essentially as described by Higgins *et al.* (1976) or Rahman *et al.* (1998). RNA was quantified by UV absorption and by separation in 1.4% (w/v) agarose-formaldehyde gels which were then visualised under UV light after staining with ethidium bromide.

## EXAMPLE 4

### Construction and screening of cDNA libraries

15 A first cDNA library, an expression cDNA library of wheat endosperm, was constructed from mRNA isolated from wheat cv Chinese Spring. RNA from 5, 7, 9, 11 and 13 days after anthesis was pooled and random primers were used for the first strand of cDNA synthesis. Monoclonal antibodies against 100 -105 kDa proteins in wheat starch granules (Rahman *et al.*, 1995) were used for immunoscreening of the expression cDNA library.

A second cDNA library was constructed from the endosperm mRNA of the hexaploid  
25 *Triticum aestivum* cultivar Wyuna, 8 - 12 days after anthesis, as described by Rahman *et al.* (1997). This library was screened with a 85-bp cDNA fragment, wSSIIP1, which was obtained by immunoscreening of the expression cDNA library as described above. The wSSIIP1 probe corresponded to nucleotide positions 988 to 1072 of wSSIIB (SEQ ID NO:1) at the hybridisation conditions as described earlier (Rahman *et al.*, 1998).

30

A third cDNA library was constructed from RNA from the endosperm of the hexaploid

*Triticum aestivum* cultivar Rosella as described by Rahman *et al.* (1997). This library was screened with a 347-bp cDNA fragment, wSSIIp1 for the first screening, and a 478-bp cDNA fragment wSSIIp3 for the second screening using the hybridisation conditions described herein.

5

### EXAMPLE 5

#### Construction and screening of *Triticum tauschii* genomic library

The genomic library used in this study, prepared from *Triticum tauschii*, var strangulata, (Accession Number CPI 110799), has been described in Rahman *et al.*,  
10 (1997). Of all the accessions of *T. tauschii* surveyed, DNA marker analysis suggests that the genome of CPI 110799 is the most closely related to the D genome of hexaploid wheat (Lagudah *et al.*, 1991).

Hybridisations were carried out in 25% formamide, 6 x SSC, 0.1% SDS at 42°C for 16  
15 hours, then filters were washed 3 times using 2 x SSC containing 0.1% SDS at 65°C for 1 hour per wash.

For the isolation of a genomic wSSII clone, the probe comprised the PCR-derived DNA fragment wSSIIp2 and positive-hybridising plaques were digested using the restriction  
20 enzyme *Bam*HI, separated on a 1% agarose gel, transferred to nitrocellulose membrane and hybridised to probe wSSIIp4 comprising nucleotides 1 to 367 of the wSSIIA cDNA clone, using the conditions described by Rahman *et al.* (1997).

For the isolation of a genomic wSSIII clone, plaques hybridising to the PCR-derived  
25 DNA fragment wSSIIIp1 from clone wSSIII.B3 (i.e. nucleotides 3620 to 3966 of SEQ ID NO:7) were selected and re-screened until plaque-purified.

### EXAMPLE 6

#### DNA sequencing and analysis

30 DNA sequencing was performed using the automated ABI system with dye terminators as described by the manufacturers. DNA sequences were analysed using the GCG

suite of programs (Devereaux *et al.*, 1984).

### EXAMPLE 7

#### DNA and RNA analysis

5 DNA was isolated and analysed as previously described (Maniatis *et al.*, 1982; Rahman *et al.*, 1998). Approximately 20  $\mu$ g of DNA was digested with restriction enzymes *Bam*HI, *Dra*I and *Eco*RI, separated on a 1% agarose gel and transferred to reinforced nitrocellulose membranes (BioRad) and hybridised with  $^{32}$ P-labelled DNA probe, either wSSIIIp1, corresponding to nucleotides 3620 to 3966 of the wheat SSIII  
10 gene, or alternatively, with the entire wSSII cDNA clone. DNA fragment probes were labelled with the Rapid Multiprime DNA Probe Labelling Kit (Promega).

The hybridisation and wash conditions were performed as described in Rahman *et al.* (1997). For RNA analysis, 10  $\mu$ g of total RNA was separated in a 1.4% agarose-  
15 formaldehyde gel and transferred to a Hybond N+ membrane (Amersham), and hybridised with cDNA probe at 42°C as previously described by Khandjian *et al.*, (1987) or Rahman *et al.*, (1998). After washing for 30 minutes at 65°C with 2x SSC, 0.1% SDS; followed by three washes of 40 minutes at 65°C with 0.2x SSC, 1% SDS, the membranes were visualised by overnight exposure at -80°C with Kodak MR X-ray  
20 film.

### EXAMPLE 8

#### Expression of wheat Sgp-1 polypeptides in the wheat endosperm

The development and use of monoclonal antibodies to the Sgp-1 proteins has been  
25 described previously (Rahman *et al.*, 1995). These antibodies were used by the present inventors to characterise the expression and localisation of the Sgp-1 proteins.

The proteins found in the matrix of the wheat starch granule are shown in Figure 1, lane 1. The remaining lanes show an immunoblot of proteins from the soluble phase  
30 (Figure 1; lanes 2-4) and the starch granule (Figure 1; lanes 5-7), respectively, following SDS-PAGE. In addition to cross-reactivity with the 100-105 kDa proteins, a

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weak cross-reaction with a 50 kDa protein in both the granule and the soluble fractions were observed (Figure 1). The Sgp-1 polypeptides are present in the starch granule throughout endosperm development (Figure 1; lanes 5-7, also see Rahman *et al.*, 1995). However, as the endosperm matures, there is a reduction in the amount of Sgp-1 protein found in the soluble fraction. Lane 4 shows that by 25 days after anthesis, the level of these proteins in the soluble fraction is substantially reduced. This observation is consistent with previous results from Rahman *et al.*, (1995), who suggested that the Sgp-1 proteins were exclusively granule bound based on studies of granules from endosperm in mid-late stages endosperm development, however, these results suggest that the partitioning of these proteins between the granule and the soluble phase changes during development.

### EXAMPLE 9

#### Isolation of cDNA clones encoding wheat starch synthase II (wSSII) proteins

Monoclonal antibodies against Sgp-1 polypeptides (Rahman *et al.*, 1995) were used to probe the expression library described in Example 4 (i.e. the first cDNA library). Three immunoreactive plaques were identified and sequenced. One clone, designated wSSIIp1, contained an 85-bp cDNA insert with homology to maize SSIIa (Harn *et al.*, 1998).

20

DNA from the wSSIIp1 clone was used as a probe in the hybridisation screening of the second cDNA library, prepared from *Triticum aestivum* cultivar Wyuna endosperm RNA as described in Example 4. Ten hybridising cDNA clones were selected and sequenced. On the basis of the DNA sequences obtained, the 10 cDNA clones can be classified into three groups. Group 1 contains 7 cDNA clones, group 2 contains 2 cDNA clones and group 3 contains 1 cDNA clone.

The longest clone from group 1 (designated wSSIIb) is 2939 bp in length (SEQ ID NO:1) and encodes a 798 -amino acid polypeptide in the region from nucleotide position 176 to nucleotide position 2569 (SEQ ID NO:2).

30

The longest clone from group 2 (designated wSSIIA) is 2842 bp in length (SEQ ID NO:3) and encodes a 799 -amino acid polypeptide in the region from nucleotide position 89 to nucleotide position 2485 (SEQ ID NO:4).

- 5 The cDNA from group 3 is a partial cDNA clone (designated wSSIID), which is 2107 bp in length (SEQ ID NO:5) and encodes a 597 -amino acid polypeptide in the region from nucleotide position 1 to nucleotide position 1791 (SEQ ID NO:6). The encoded polypeptide is approximately a 200 amino acid residues shorter than that of polypeptides encoded by longest clones of group 1 or 2 clones, respectively (Figure 10 2).

Comparison of the three cDNA clones, wSSIIB, wSSIIA and wSSIID shows that they share 95.7% to 96.6% identity at the amino acid level, with variation at 44 amino acid positions between the three sequences (Figure 3). Of the 44 amino acid changes 15 between these sequences, 31 changes occur in the N-terminal region (residues 1 to 300), 10 changes occur in the central region (residues 301 to 729) and 3 changes occur in the C-terminal region (residues 730 to 799). The wSSIIA polypeptide (799 amino acid residues) and wSSIIB polypeptide (798 amino acid residues) sequences differ in length by a single amino acid residue, due to the deletion of Asp-69 from the 20 wSSIIB polypeptide sequence.

A comparison of the nucleotide sequences of the wSSIIA, wSSIIB and wSSIID cDNA clones with the nucleotide sequence of the wSSIIP1 cDNA obtained by immunoscreening confirms that the wSSIIP1 sequence is found in each cDNA (Figure 25 3). The peptide encoded by the wSSIIP1 cDNA clone corresponds to amino acid residues in the region from residue 272 to residue 298 of the wSSIIA polypeptide, and to amino acid residues in the region from residue 271 to residue 297 of the wSSIIB polypeptide (see Figure 3). Thus, the peptide epitope encoded by wSSIIP1 that reacts with the anti-Sgp-1 monoclonal antibodies can therefore be localised to this region of 30 the wSSIIA and wSSIIB polypeptides and to the corresponding region of the wSSIID polypeptide.



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Notwithstanding that a region having about 63% amino acid sequence identity to the peptide epitope encoded by clone wSSIIp1 is found in the maize SSIIa polypeptide (Figure 3), the degree of amino acid conservation between maize and wheat sequences in this region of the polypeptide is insufficient for immunological cross-reactivity to occur between these species using the monoclonal antibodies to the wheat Sgp-1 proteins described by Rahman *et al.* (1995). Additionally, this peptide epitope is not found in granule-bound starch synthases, SSI, or SSIII (data not shown).

The wSSIIIB cDNA (SEQ ID NO:1) encodes an amino acid sequence comprising the peptide motif AAGKKDAGID (SEQ ID NO: 18) between residues 60 and 69 of SEQ ID NO:2 (Figure 3) which, with the exception of the second residue, is identical to the N-terminal of the 100 kDa (A<sup>T</sup>/L GKKDAGID: SEQ ID NOS:19 and 20) protein (Sgp-B1) from the wheat starch granule (note that the sequence given in Rahman *et al.*, 1995 (A<sup>T</sup>/L GKKDAL: SEQ ID NOS: 21 and 22 ) has been revised following further amino acid sequence analysis).

The wSSIIA cDNA clone (SEQ ID NO:3) encodes an amino acid sequence comprising the peptide motif AAGKKDARVDDDDAA (SEQ ID NO: 23) at residues 60 to 73 of SEQ ID NO:4, which is about 66% identical to the N-terminal amino acid sequence (i.e. ALGKKDAGIVDGA: SEQ ID NO: 24) of the 104 kDa and 105 kDa starch granule proteins, Sgp-D1 and Sgp-A1 respectively, as determined by sequence analysis of isolated protein (Rahman *et al.*, 1995).

Furthermore, Takaoka *et al.* (1997) reported the amino acid sequences of 3 polypeptides obtained from sequencing starch granule proteins derived from the Sgp-1 proteins. Peptide 3 described by Takaoka *et al.* (1997) corresponds to amino acid residues 378 to 387 of the amino acid sequence of the wSSIIA cDNA (SEQ ID NO:4; Figure 3). Peptides 1 and 2 described by Takaoka *et al.* (1997) could not be detected in the amino acid sequences of the wSSII cDNA clones of the present invention, however peptide 1 of Takaoka *et al.* (1997) can be found in the amino acid sequences of SSI from maize, rice, wheat and potato (data not shown).



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Denyer *et al.* (1995) demonstrated that the Sgp-1 proteins possess starch synthase activity and, as a consequence, the wSSIIB, wSSIA and wSSIID cDNA clones encode starch synthase enzymes that are differentially expressed in a developmentally-regulated manner in both the soluble and granule-bound fractions of the endosperm (Figure 1). Based on the nomenclature suggested by Harn *et al.* (1998), it is appropriate to describe the Sgp-1 proteins as "starch synthases" rather than "granule-bound starch synthases".

### EXAMPLE 10

#### 10                    **Analysis of wheat starch synthase II mRNA expression**

The mRNA for wheat starch synthase II could be detected in leaves, pre-anthesis florets and endosperm of wheat when total RNAs isolated from these tissue were probed with a PCR probe, wSSIIP2, corresponding to nucleotide positions 1435 to 1835 bp of wSSIIB-cDNA (SEQ ID NO:1; Figure 4). Unlike wSSI, which could not be detected in wheat leaves derived from plants grown under the same conditions, wSSII genes are highly-expressed in the leaves (Figure 4, lane 1), and expressed at an intermediate level in pre-anthesis florets (Figure 4, lane 2), and at much lower levels in developing wheat endosperm cells (Figure 4, lanes 3-11). In contrast, the maize SSIIa is expressed predominantly in the endosperm, whilst the maize SSIIb is detected mainly in the leaf, albeit at low levels (Harn *et al.*, 1998).

The wSSII mRNA was detectable in the endosperm 6 days after anthesis and mRNA levels increase between 8 and 18 days post-anthesis, after which time levels of mRNA decline.

25

Southern blotting experiments in wheat demonstrated that the wSSIIP2 probe used detected only a single copy of the SSII gene in each genome (data not shown). Thus, it is unlikely that this probe cross-hybridised with mRNAs encoded by genes other than wSSII.

30

## EXAMPLE 11

### Chromosomal localization of the wheat wSSII genes.

#### I. Amplification of specific cDNA regions of wheat starch synthase II using PCR

Two PCR products, wSSIIp2 and wSSIIp3 were amplified from the cDNA clone wSSIIb  
5 and used for the northern hybridisation and Southern hybridisation, respectively.

The primers sslIa (5' TGTTGAGGTTCCATGGCACGTTC 3': SEQ ID NO: 25) and sslIb  
(5' AGTCGTTCTGCCGTATGATGTCG 3': SEQ ID NO: 26) were used to amplify the  
cDNA fragment wSSIIp2 (i.e. nucleotide positions 1435 to 1835 of SEQ ID NO:1).

10

The primers sslIc (5' CCAAGTACCAGTGGTGAACGC 3': SEQ ID NO: 27) and sslId  
(5' CGGTGGGATCCAACGGCCC 3': SEQ ID NO: 28) were used to amplify the cDNA  
fragment wSSIIp3 (i.e. nucleotide positions 2556 to 2921 of SEQ ID NO:1).

15 The amplification reactions were performed using a FTS-1 thermal sequencer (Corbett,  
Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for  
1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute.

#### II. PCR and nucleotide sequence analysis of 3' sequences of wheat SSII genes

20 Genomic DNA was extracted from wild-type Chinese Spring wheat, and from three  
nullisomic-tetrasomic lines of chromosome 7 of Chinese Spring wheat, and from  
*Triticum tauschii* (var strangulata, accession number CPI 100799), and used as a  
template for the amplification and nucleotide sequence analysis of wheat SSII genes.

25 RFLP analysis of *Bam*HI and *Eco*RI restricted DNA from each wheat or *T. Tauschii* line  
was carried out using the wSSIIp3 fragment as a probe. Three hybridising bands were  
obtained which could be assigned to chromosomes 7A, 7B and 7D, respectively (data  
not shown). This analysis indicates that there is a single copy of the wSSII gene in  
each genome in hexaploid wheat, consistent with the findings of Yamamori and Endo  
30 (1996) who located the SGP-A1, B1 and D1 proteins to the short arm of chromosome  
7.

PCR analysis was used to assign each of the cDNA clones to the individual wheat genomes. A single 365 bp PCR fragment was obtained from nullisomic-tetrasomic genomic DNA of Chinese Spring when primers sslc and ssld were used for the PCR amplification (Figure 5, right panel). This PCR product is obtained only from lines 5 bearing the B genome. The fragment was cloned and sequenced and shown to be identical to a 365 bp region of the wSSlIB cDNA. An identical fragment is obtained by PCR amplification of the wSSlIB cDNA clone, but not by amplification of the wSSIIA or wSSIID clones, supporting the conclusion that the wSSlIB cDNA is the product of a gene located on chromosome 7 of the B genome of hexaploid wheat.

10

Two PCR products were also amplified from nullisomic-tetrasomic genomic DNA of Chinese Spring using the primers sslc and ssle (Figure 5, left panel). One PCR fragment, approximately 350 bp is only amplified when the A genome is present, and a second 322 bp product is only amplified when the D-genome is present. The 350 and 15 322 bp PCR products were also cloned and sequenced and shown to be identical to the wSSIIA and wSSIID cDNAs, respectively, supporting the conclusion that the wSSIIA and wSSIID cDNAs are the products of genes located on chromosomes 7A and 7D, respectively.

20

## EXAMPLE 12

### Isolation of genomic wSSII clones

Screening of a genomic library from the D-genome donor of wheat, *T. tauschii*, was performed as described in Example 5, using the PCR-derived DNA fragment wSSIIp2 as a hybridisation probe. A positive-hybridising clone, designated wSSII-8, and 25 comprising a putative *T. tauschii* homologue of the wSSII gene, was isolated.

Positive-hybridising plaques were digested using the restriction enzyme *Bam*HI, separated on a 1% agarose gel, transferred to nitrocellulose membrane and hybridised to probe wSSIIp4 comprising nucleotides 1 to 367 of the wSSIIA cDNA clone, using 30 the conditions described by Rahman *et al.* (1997). Clone wSSII-8 also hybridises strongly to the wSSIIp4 probe, confirming its identity as a genomic wSSII gene.

The complete nucleotide sequence of the wSSII gene was determined and is presented herein as SEQ ID NO: 37. The structural features of this gene are present in Table 3. A schematic representation of the intron/exon organisation of this gene is also presented in Figure 6.

5

TABLE 3

**Structural features of the wheat starch synthase II genomic gene**

	Nucleotide Position in SEQ ID NO: 37	Feature	Length (bases)
10	1- 1416	5'-untranscribed region and promoter sequence	1416
	1417 - 1743	exon 1	327
	1480-1482	translation start codon (ATG)	3
	1744 - 1847	intron 1	104
	1848 - 2553	exon 2	706
15	2554 - 2641	intron 2	88
	2642 - 2706	exon 3	65
	2707 - 3606	intron 3	900
	3607 - 3684	exon 4	78
	3685 - 3773	intron 4	89
20	3774 - 3884	exon 5	111
	3885 - 3981	intron 5	97
	3982 - 4026	exon 6	45
	4027 - 4406	intron 6	380
	4407 - 4580	exon 7	174
25	4581 - 7296	intron 7	2716
	7297 - 8547	exon 8	1251
	8251 - 8253	translation stop codon (TGA)	3
	8548 -9024	3'-untranscribed region	477

### EXAMPLE 13

**Cloning of specific cDNA regions of wheat starch synthase III using RT-PCR**  
PCR primers were used to amplify sequences of starch synthase III from wheat endosperm cDNA. The design of PCR primers was based on the sequences of starch  
5 synthase III from potato and the *du1* starch synthase III gene of maize.

First-strand cDNAs were synthesised from 1  $\mu$ g of total RNA (derived from endosperm of the cultivar Rosella, 12 days after anthesis) as described by Maniatis *et al.* (1982), and then used as templates to amplify two specific cDNA regions, wSSIIIp1 and  
10 wSSIIIp2, of wheat starch synthase III by PCR.

The primers used to obtain the cDNA clone wSSIIIp1 were as follows:

Primer wSS3pa (5' GGAGGTCTTGGTGATGTTGT 3': SEQ ID NO: 29); and

Primer wSS3pb (5' CTTGACCAATCATGGCAATG 3': SEQ ID NO: 30).

15

The primers used to obtain the cDNA clone wSSIIIp2 were as follows:

Primer wSS3pc (5' CATTGCCATGATTGGTCAAG 3': SEQ ID NO: 31); and

Primer wSS3pd (5' ACCACCTGTCCGTTCCGTTGC 3': SEQ ID NO: 32).

20 The amplified clones wSSIIIp1 and wSSIIIp2 were used as probes to screen the third cDNA library and *T. tauschii* genomic DNA library as described in Example 4.

A further probe designated wSSIIIp3 was used for screening the third cDNA library, as described in Example 4. Probe wSSIIIp3 was amplified by PCR from a cDNA clone  
25 produced from the first screening using the following amplification primers:

Primer wSS3pe (5' GCACGGTCTATGAGAACAATGGC 3': SEQ ID NO: 33); and

Primer wSS3pf (5' TCTGCATACCACCAATCGCCG 3': SEQ ID NO: 34).

The amplification reactions were performed using a FTS-1 or FTS4000 thermal  
30 sequencer (Corbett, Australia) for 1 cycle of 95°C for 2 minutes; 35 cycles of 95°C for 30 seconds, 60°C for 1 minutes, 72°C for 2 minutes and 1 cycle of 25°C for 1 minute.



Amplified sequences of the expected length were obtained, cloned and sequenced, and shown to contain DNA sequences highly homologous to the maize and potato SSIII genes. PCR fragments were subsequently used to probe a wheat cDNA library  
5 by DNA hybridisation and 8 positive clones were obtained, including one 3 kb cDNA. A region from the 5' end of this cDNA was amplified by PCR and used a probe for a second round of screening the cDNA library, obtaining 8 cDNA clones. Of these, one cDNA was demonstrated to be full length (wSSIII.B3, 5.36 kb insert). The sequence of the 5,346 bp wSSIII.B3 cDNA clone is given in SEQ ID NO:7.

10

Sequencing of the 8 cDNA clones obtained from the second round screening of the wheat cDNA library revealed that there were at least 2 classes of cDNA encoding SSIII present, possibly being encoded by homeologous genes on different wheat genomes. The sequence of a representative of this second class of cDNA clones, wSSIII.B1, is  
15 shown in SEQ ID NO:9. The 3261 bp clone wSSIII.B1 is not full length, however it is similar to nucleotides 1739 to 5346 of the homeologous clone wSSIII.B3 (SEQ ID NO: 7). Clone wSSIII.B1 has an open reading frame between nucleotide positions 1 and 3177.

20 An open reading frame is found in the cDNA clone wSSIII.B3 (SEQ ID NO:7), in the region between position 29, commencing the ATG start codon, and nucleotide position 4912. The amino acid sequence deduced from this open reading frame is shown in SEQ ID NO:8.

25 An alignment of the deduced amino acid sequences of SSIII from maize, potato and wheat is shown in Figure 7. There is about 56.6% identity between the maize SSIII and wheat wSSIII.B3 sequence at the amino acid level.

The C-terminal domain of starch synthases comprise the catalytic domain, and a  
30 characteristic amino acid sequence motif KVGGLGDVVTSLSRVQDLGHNVEV (SEQ ID NO: 35) in maize, or alternatively KVGGLGDVVTSLSRVQDLGHTVEV (SEQ ID

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NO: 36) in wheat, marking the first conserved region in the C-terminal domain. This amino acid sequence is present at amino acid residues 1194 to 1218 of SEQ ID NO: 8.

5 The amino acid identity between maize dull1 and wSSIII.B3 in the N-terminal region (i.e. amino acids 1 to 600 in Figure 7) is only 32.2%; whilst the amino acid identity in the central region (i.e. amino acids 601 to 1248 in Figure 7) is 68.4%; and in the C-terminal region (i.e. amino acids 1249 to 1631 in Figure 7) is 84.6%. Accordingly, the SSIII starch synthases are much more highly conserved between maize and wheat in  
10 the region comprising the catalytic domain of the proteins.

#### EXAMPLE 14

##### Analysis of wheat starch synthase III mRNA expression

Figure 8 shows the expression of wSSIII mRNA during endosperm development in two  
15 wheat varieties grown under defined environmental conditions. The expression of the gene is seen very early in endosperm development in both cultivars, 4 days after anthesis (Figure 8, panels a and b). Expression in the leaf of the variety Gabo is very weak (Figure 8, panel c, Lane L) whereas strong expression is seen in pre-anthesis florets (Figure 8, panel c, Lane P).

20

#### EXAMPLE 15

##### Amino acid sequence comparisons between wheat SSII and SSIII polypeptides

Amino acid sequence comparisons between wheat BSSS, SSI, SSII and SSIII  
25 polypeptides reveals eight highly-conserved domains (Figure 9). The amino acid sequences of these domains are represented in the wheat SSIII amino acid sequence by the following sequence motifs:

- (a) Region 1: KVGGLGDVVT;
- (b) Region 2: GHTVEVILPKY;
- 30 (c) Region 3: HDWSSAPVAWLYKEHY;
- (d) Region 4: GILNGIDPDIWDPYTD;

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- (e) Region 5: DVPIVGIITRLTAQKG;
- (f) Region 5a: NGQVVLLGSA;
- (g) Region 6: AGSDFIIVPSIFPCGLTQLVAMRYGS; and
- (h) Region 7: TGGLVDTV.

5

These conserved amino acid sequences are summarised in Table 4. As shown in Table 4 below, there is at least about 25% amino acid sequence identity, preferably at least about 30% amino acid sequence identity, more preferably at least about 35% amino acid sequence identity, more preferably at least about 40% amino acid sequence identity, more preferably at least about 45% amino acid sequence identity, more preferably at least about 50% amino acid sequence identity, more preferably at least about 55% amino acid sequence identity, more preferably at least about 60% amino acid sequence identity, more preferably at least about 65% amino acid sequence identity, more preferably at least about 70% amino acid sequence identity, more preferably at least about 75% amino acid sequence identity, more preferably at least about 80% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity and even more preferably at least about 95% amino acid sequence identity between the amino acid sequences of plant starch synthase enzymes, in particular wheat starch synthases.

From the data presented in Table 4, the most conserved regions of the wheat SSII and SSIII polypeptides are a region of 6 or 7 identical amino acids in Region 1 and a region of 8 or 9 identical amino acids in Region 6. The lowest regions of identity are found in regions 3 and 5a.

For each of the amino acid sequences presented in the first column of Table 4, which are specific for wSSIII polypeptides, corresponding signature motifs which are specific for wSSII-A, wSSII-B, and wSSII-D polypeptides can be derived from the alignment, as follows:

Region 1: KTGGLGDVAGA;

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- 5           Region 2:    GHRVMVVVPRY;  
          Region 3:   NDWHTALLPVYLKAYY;  
          Region 4:   GIVNGIDNMEWNPEVD;  
          Region 5:   DVPLLGFGRDLGQKG;  
          Region 5a:  DVQLVMLGTG;  
          Region 6:   AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and  
          Region 7:   VGG(V/L)RDTV.

Comparison of the amino acid sequences of all available starch synthases with the  
10 deduced amino acid sequences of the three wSSII cDNA clones of the present  
invention (i.e. wSSIIb, wSSIIa and wSSIID) was conducted using PILEUP analysis  
(Devereaux *et al.*, 1984) and data are presented herein as a dendrogram (Figure 10).  
The sequence of the glycogen synthase of *E. coli* was also included. Based upon their  
amino acid similarities, four classes of plant starch synthases can be defined: GBSS,  
15 SSI, SSII and SSIII.

Table 5 shows that levels of identity at the amino acid level between the wSSII  
sequences, as determined using the BESTFIT programme in GCG (Devereaux *et al.*,  
1984), and other class II starch synthases range from 70% identity with potato SSII to  
20 85% identity with maize SSIIa. Both wSSIIb and wSSIID showed significantly higher  
homology to maize SSIIa than wSSIIa. Based upon sequence identities and the  
function of the Sgp-1 proteins in wheat, the wSSIIb, wSSIIa and wSSIID cDNA clones  
are members of the starch synthase II (SSII) group and are more similar in sequence  
to maize SSIIa than maize SSIIb.

25

TABLE 4

Identities between conserved motifs of plant starch synthases

	Sequence in wSSIII polypeptide	Number of conserved residues between wheat starch synthases	Number of conserved residues between wheat SSII and SSIII polypeptides
5	Region 1: KVGGLGDVWTS	6/11 residues	6/11 residues
	Region 2: GHTVEVILPKY	6/11 residues	6/11 residues
10	Region 3: HDWSSAPVAWLYKEHY	4/16 residues	5/16 residues
	Region 4: GILNGIDPDIWDPYTD	7/16 residues	8/16 residues
	Region 5: DVPIVGIITRLTAQKG	8/16 residues	10/16 residues
15	Region 5a: NGQVVLLGSA	4/10 residues	4/10 residues
	Region 6: AGSDFIIVPSIFPCGLT QLVAMRYGS	15/27 residues	17/27 residues
20	Region 7: TGGLVDTV	5/9 residues	5/9 residues



TABLE 5

	wSSII-A	wSSII-B	wSSII-D
wSSI-A	100%		
wSSII-B	95.9%	100%	
5 wSSII-D	96.3%	96.7%	100%
maize SSIIa	76.1%	85.2%	84.7%
maize SSIIb	76.3%	76.7%	75.9%
pea SSII	72.0%	72.2%	71.8%
10 potato SSII	70.9%	71.1%	70.3%

Figure 11 shows a schematic representation of an alignment of plant starch synthase sequences, including wheat GBSS, wheat SSI, wheat SSII-A1, maize SSIIa, and maize dull-1 polypeptides, in which the position of the first homologous region, comprising the consensus motif KXGG, is used as the basis of the alignment. The major differences in structure between the classes of genes are found in the length of the N-terminal region between the transit peptide and the first conserved region. At one extreme, the GBSS genes have a very short N-terminal arm, whereas the *du1* starch synthase contains a very long N-terminal extension containing several distinct regions. The wSSII genes contain an N-terminal extension which is longer than either GBSS, SSI, or SSIIb, and slightly longer than the maize SSIIa gene.

### EXAMPLE 16

#### Isolation of genomic clones for SSIII

Screening of a genomic library from the D-genome donor of wheat, *T. tauschii*, identified a number of clones which hybridised to the wSSIII PCR fragment. Positive plaques in the genomic library were selected as those hybridising with a probe that had been generated by PCR (amplifying between nucleotide positions 3620 to 3966) from the SSIII cDNA as template. The primer sequences used were as follows:

wSS3pa (5' GGAGGTCTTGGTGATGTTGT 3': SEQ ID NO: 29); and

30 wSS3pb (5' CTTGACCAATCATGGCAATG 3' : SEQ ID NO: 30).

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Hybridisation was carried out in 25% formamide, 6 x SSC, 0.1% SDS at 42 °C for 16 hour, then washed three times with 2 x SSC containing 0.1% SDS at 65 °C, for 1 hour per wash. shows an example of a plaque lift showing positive and negative hybridisations for plaques containing the *T. tauschii* homologue of the wSSIII.B3 gene.

5 DNA was isolated from positive-hybridising  $\lambda$  clones using methods described by Maniatis *et al.* Briefly, DNA was digested using *Bam*HI or *Bgl*II and sub-cloned in to the vector pJKKmfm. DNA sequencing was performed using the automated ABI system with dye terminators as described by the manufacturers. DNA sequences were  
10 analysed using the GCG suite of programs (Devereaux *et al.*, 1984).

Nucleotide sequences of the genomic SSIII clone from *T. tauschii* are provided herein as 6 contiguous sequences designated fragments 1 to 6 (SEQ ID NOs: 11 to 16, respectively). Table 6 defines the relative positions of these fragments with respect to  
15 the SSIII cDNA and describes the positions of exons. Figure 11 shows this information schematically.

The complete nucleotide sequence of a wheat SSIII genomic gene is presented herein as SEQ ID NO: 38. The structural features of this gene are presented in Table 7. A  
20 schematic representation of the intron/exon organisation of this gene is also presented in Figure 12.

## EXAMPLE 17

### Discussion

25 Early work on the Sgp-1 starch synthase proteins (Denyer *et al.*, 1995; Rahman *et al.*, 1995) was based on the localisation of these proteins in the wheat starch granule, and no definitive conclusion concerning their presence or absence in soluble extracts of the wheat endosperm was presented.

30 We have now demonstrated that a monoclonal antibody against the Sgp-1 proteins cross reacts strongly with those starch synthase proteins having apparent molecular

weights of 100-105 kDa in soluble extracts, however, the appearance of these proteins in soluble extracts is dependant on the developmental stage of the endosperm material. Whilst the proteins can be detected in the soluble phase in early to mid endosperm development, little or no soluble protein remains in late endosperm development (Figure 1). This observation accounts for the failure of Rahman *et al.* (1995) to detect the protein in soluble extracts in a previous report.

Based upon the localisation of the Sgp-1 starch synthase proteins in the wheat endosperm, the following nomenclature is suggested for wheat starch synthase enzymes: wGBSS for the 60 kDa granule bound starch synthase (Wx); wSSI for the 75 kDa starch synthase I (Sgp-3); wSSII for the 100 - 105 kDa proteins (Sgp-1); and wSSIII for a soluble high molecular starch synthase.

The present invention provides cDNA and genomic clones encoding the wSSII and wSSIII polypeptides and the corresponding genomic clones. Whilst the evidence is compelling that the wSSIIA, wSSIIB and wSSIID cDNAs encode the Sgp-A1, Sgp-B1 and Sgp-D1 proteins of the wheat starch granule, molecular weights calculated from the deduced amino acid sequences of the clones are considerably lower than estimates obtained from SDS-PAGE. The molecular weight of the precursor wSSIIA protein is 87,229 Da, and the mature protein 81,164 Da, yet the estimated molecular weight in our experience is 105 kDa. The assignment of the wSSIIA cDNA to the A-genome of wheat is demonstrated in Figure 5, and the assignment of the 105 kDa protein to the A-genome in Denyer *et al.* (1995) and Yamamori and Endo (1996). Similarly, the molecular weight of the wSSIIB protein is 86,790 Da and the mature protein 80,759 Da, yet the molecular weight of the Sgp-B1 protein is estimated to be 100 kDa. No comparison can be made of the wSSIID sequences as a full length cDNA clone was not obtained. The wSSIIA and wSSIIB amino acid sequences differ by just a single amino acid residue, yet there is an apparent difference of 5 kDa in molecular weight when estimated by SDS-PAGE. Several possibilities can be advanced to account for this apparent discrepancy in molecular weights. Firstly, the wSSII proteins may not migrate in SDS-PAGE in accordance with their molecular weight because they

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retain some conformation under the denaturing conditions used. Secondly, the proteins may be glycosylated. It is also possible that the proteins may be non-covalently linked to starch through a high affinity starch binding site which survives denaturation and SDS-PAGE. Differences between the apparent molecular weights and those calculated  
5 from the deduced amino acid sequences will have to be defined in establishing the relationship between the wSSII proteins and proteins encoded by the analogous SSII genes of other species.

The catalytic domain of the starch synthases is found at the C-terminal end of the  
10 protein (Gao *et al.*, 1998; Ham *et al.*, 1998). Ham *et al.* (1998) identified 7 conserved regions among SSIIa, SSIIb, SSI and GBSS sequences. We have identified an additional conserved region (designated region 5a in Table 4 and Figure 10) comprising the amino acid sequence motif DVQLVMLGTG, by a comparison of the wSSII and wSSIII sequences of the present invention with differing isoforms of other  
15 plant starch synthases (GBSS, SS1, SSII and SSIII). The conservation of eight peptide regions among the 4 classes of starch synthases is striking, in terms of their sequence homologies and their alignment.

Analysis of the wheat SSII genes shows that there is a motif, PVNGENK, which is  
20 repeated. The area surrounding the repeated PVNGENK motif is not homologous to maize SSIIa and the insertion of this region is responsible for the difference in length between the wheat SSII and maize SSIIa genes. In pea and potato SSII polypeptides, a PPP motif (Figure 3; residues 251-253 and 287-289 respectively) has been suggested to mark the end of the N-terminal region and to facilitate the flexibility of an  
25 "N-terminal arm". This motif is not found in either the maize or wheat SSII sequences.

The generation of a wheat line combining null alleles at each of the three wSSII loci, wSSIIA, wSSIIB and wSSIID, has been reported recently by Yamamori (1998). In this triple null line, the large starch granules were reported to be mostly deformed and a  
30 novel starch with high blue value was observed when stained with iodine, indicating that wSSII is a key enzyme for the synthesis of starch in wheat. Further analysis of the

starch derived from this triple null mutant is in progress.

Mutations in starch synthases are known in three other species. In pea, mutation in SSII gives rise to starch with altered granule morphology and an amylopectin which  
5 yields an oligosaccharide distribution with reduced chain length on debranching, compared to the wild type (Craig *et al.*, 1998). A similar mutation in a gene designated SSII is known in *Chlamydomonas* (the *sta-3* mutation) and similar effects on granule morphology and amylopectin structure are observed (Fontaine *et al.*, 1993). In maize, two mutations affecting starch synthases are known. First, the *dull1* mutation has been  
10 shown to be caused by a lesion within the *du1* SSIII-type starch synthase gene (Gao *et al.*, 1998). A second mutation, the *sugary-2* mutation yields a starch with reduced amylopectin chain lengths on debranching (this mutation co-segregates with the SSIIa locus (Harn *et al.*, 1998) although direct evidence that the *sugary-2* mutation is caused by a lesion in the SSIIa gene is lacking). In the SSII mutants of each of these species,  
15 amylose biosynthesis capacity is retained, suggesting different roles in amylose and amylopectin synthesis for the GBSS and SSII genes. Given the conservation in overall organisation of the GBSS and SSII genes (see Figures 12 and 13), when an alignment is made based on the KTGGL motif of the first conserved region, this focuses attention on the role(s) of the N-terminal region in defining substrate specificity and the  
20 localisation of the proteins as the N-terminal region is the major area of divergence between the 4 classes of starch synthases. However, it is premature to exclude the influence of more subtle mutations in central and C-terminal regions of the gene.

The cloning of the wSSII and wSSIII cDNAs and genomic clones described herein  
25 provides useful tools for the further study of the roles of the starch synthases in wheat. Firstly, they provide a source of markers which can be used to recover and combine null or divergent alleles. Secondly, genetic manipulation of wheat by gene suppression or over-expression can be carried out, and the genes may be used for over expression in other species. The promoter regions of these genes are also useful in regulating the  
30 expression of starch synthase genes and other heterologous genes in the developing wheat endosperm and in pre-anthesis florets of wheat.



**TABLE 6**  
**Summary of the Wheat Starch Synthase III Genomic Sequence**

Fragment in genomic DNA clone	Length (bp)	Features in SEQ ID NOS:11 to 16	Corresponding region in cDNA sequence
Fragment 1 (SEQ ID NO: 11)	728	Translation start codon (nucleotides 287 to 289); Exon 1.1 (nucleotides 260 to 385).	Exon 1.1: nucleotides 1 to 126
Fragment 2 (SEQ ID NO: 12)	2446	Exon 2.1 (nucleotides 1 to 1938); Exon 2.2 (nucleotides 2197 to 2418).	Exon 2.1: nucleotides 1008 to 2948; Exon 2.2: nucleotides 2949 to 3171
Fragment 3 (SEQ ID NO: 13)	1032	Exon 3.1 (nucleotides 310 to 580)	Exon 3.1: nucleotides 3172 to 3440
Fragment 4 (SEQ ID NO: 14)	892	Exon 4.1 (nucleotides 678 to 853)	Exon 4.1: nucleotides 3441 to 3616
Fragment 5 (SEQ ID NO: 15)	871	Partial Exon 5.1 (nucleotides 1 to 29) Exon 5.2 (nucleotides 293 to 463) Exon 5.3 (nucleotides 589 to 695)	Exon 5.1: nucleotides 3908 to 3937 (partial) Exon 5.2: nucleotides 3938 to 4108 Exon 5.3: nucleotides 4109 to 4215
Fragment 6 (SEQ ID NO: 16)	1583	Exon 6.1 (nucleotides 471 to 653); Exon 6.2 (nucleotides 770 to 902); Exon 6.3 (nucleotides 999 to 1110); Exon 6.4 (nucleotides 1201 to 1328); Partial Exon 6.5 (nucleotides 1408 to 1583); Translation stop codon (nucleotides 1536 to 1538)	Exon 6.1: nucleotides 4238 to 4420 Exon 6.2: nucleotides 4421 to 4552 Exon 6.3: nucleotides 4553 to 4664 Exon 6.4: nucleotides 4665 to 4793 Exon 6.5: nucleotides 4794 to 4966 (partial)

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**TABLE 7**  
**Structural features of the wheat starch synthase III genomic gene**

	Nucleotide Position in SEQ ID NO: 38	Feature	Length (bases)
5	1- 973	5'-untranscribed region and promoter sequence	973
	974 - 1099	exon 1	126
	1001-1003	translation start codon (ATG)	3
	1100 - 2056	intron 1	957
	2057 - 2120	exon 2	64
10	2121 - 2588	intron 2	468
	2589 - 5291	exon 3	2703
	5292 - 5549	intron 3	258
	5550 - 5767	exon 4	218
	5768 - 6103	intron 4	336
15	6104 - 6374	exon 5	271
	6375 - 7148	intron 5	774
	7149 - 7324	exon 6	176
	7325 - 7438	intron 6	114
	7439 - 7546	exon 7	108
20	7547 - 7792	intron 7	246
	7793 - 7902	exon 8	110
	7903 - 8797	intron 8	895
	8798 - 8900	exon 9	103
	8901 - 9164	intron 9	264
25	9165 - 9335	exon 10	171
	9336 - 9460	intron 10	125
	9461 - 9589	exon 11	129
	9590 - 9677	intron 11	88

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	9678 - 9860	exon 12	183
	9861 - 9977	intron 12	117
	9978 - 10109	exon 13	132
	10110 - 10205	intron 13	96
5	10206 - 10317	exon 14	112
	10318 - 10407	intron 14	90
	10408 - 10536	exon 15	129
	10537 - 10618	intron 15	82
	10619 - 11146	exon 16	128
10	10744 - 10746	translation stop codon (TGA)	3
	11147 - 11611	3'-untranscribed region	465

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**CLAIMS:**

1. An isolated nucleic acid molecule which comprises a sequence of nucleotides selected from the group consisting of:
  - (i) the nucleotide sequence set forth in SEQ ID NO: 1 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (ii) the nucleotide sequence set forth in SEQ ID NO: 3 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (iii) the nucleotide sequence set forth in SEQ ID NO: 5 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (iv) the nucleotide sequence set forth in SEQ ID NO: 7 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (v) the nucleotide sequence set forth in SEQ ID NO: 9 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (vi) the nucleotide sequence set forth in SEQ ID NO: 11 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (vii) the nucleotide sequence set forth in SEQ ID NO: 12 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (viii) the nucleotide sequence set forth in SEQ ID NO: 13 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (ix) the nucleotide sequence set forth in SEQ ID NO: 14 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (x) the nucleotide sequence set forth in SEQ ID NO: 15 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (xi) the nucleotide sequence set forth in SEQ ID NO: 16 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (xii) the nucleotide sequence set forth in SEQ ID NO: 37 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;
  - (xiii) the nucleotide sequence set forth in SEQ ID NO: 38 or the protein-encoding

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region thereof or a degenerate nucleotide sequence thereto;

(xiv) the nucleotide sequence set forth in SEQ ID NO: 11 or the protein-encoding region thereof or a degenerate nucleotide sequence thereto;

(xv) a nucleotide sequence which encodes a wheat starch synthase polypeptide as hereinbefore defined wherein said nucleotide sequence has at least about 85% identity overall to any one of (i) to (xiv); and

(xvi) a nucleotide sequence which is complementary to any one of (i) to (xv).

2. The isolated nucleic acid molecule according to claim 1 wherein the wheat starch synthase polypeptide further comprises one or more amino acid sequences selected from the group consisting of:

(a) KVGGLGDVVT;

(b) GHTVEVILPKY;

(c) HDWSSAPVAWLYKEHY;

(d) GILNGIDPDIWDPYTD;

(e) DVPIVGIITRLTAQKG;

(f) NGQVLLGSA;

(g) AGSDFIIVPSIFEPCGLTQLVAMRYGS;

(h) TGGLVDTV;

(i) KTGGLGDVAGA;

(j) GHRVMVVVPY;

(k) NDWHTALLPVYLKAYY;

(l) GIVNGIDNMEWNPEVD;

(m) DVPLLGFGRLDGQKG;

(n) DVQLVMLGTG;

(o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and  
(p)VGG(V/L)RDTV.

3. The isolated nucleic acid molecule according to claim 2 wherein the wheat starch synthase polypeptide comprises at least three of said amino acid sequences selected from the group consisting of (a) to (h).
4. The isolated nucleic acid molecule according to claim 2 wherein the wheat starch synthase polypeptide comprises at least six of said amino acid sequences selected from the group consisting of (i) to (p).
5. The isolated nucleic acid molecule according to claim 1 encoding a wheat starch synthase II polypeptide.
6. The isolated nucleic acid molecule according to claim 1 encoding a wheat starch synthase III polypeptide.
7. An isolated nucleic acid molecule encoding a starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:
  - (a) GHTVEVILPKY;
  - (b) HDWSSAPVAWLYKEHY;
  - (c) DVPIVGIITRLTAQKG;
  - (d) NGQVVLLGSA;
  - (e) AGSDFIIVPSIFPCGLTQLVAMRYGS;
  - (f) TGGLVDTV;
  - (g) GIVNGIDNMEWNPEVD; and

(h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.

8. The isolated nucleic acid molecule of claim 5 encoding a wheat starch synthase II polypeptide which comprises an amino acid sequence selected from the group consisting of:
  - (i) SEQ ID NO: 2;
  - (ii) SEQ ID NO: 4;
  - (iii) SEQ ID NO: 6; and
  - (iv) a homologue of any one of (i) to (iii) having at least about 85% identity thereto.
9. The isolated nucleic acid molecule of claim 6 encoding a wheat starch synthase III polypeptide which comprises an amino acid sequence selected from the group consisting of:
  - (i) SEQ ID NO: 8;
  - (ii) SEQ ID NO: 10; and
  - (iii) a homologue of (i) or (ii) having at least about 85% identity thereto.
10. A probe or primer comprising at least about 15 contiguous nucleotides in length derived from the nucleotide sequence according to claim 1.
11. The probe or primer according to claim 10 comprising a nucleotide sequence selected from the group consisting of:
  - (i) the nucleotide sequence set forth in SEQ ID NO: 25;
  - (ii) the nucleotide sequence set forth in SEQ ID NO: 26;
  - (iii) the nucleotide sequence set forth in SEQ ID NO: 27;



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- (iv) the nucleotide sequence set forth in SEQ ID NO: 28;
- (v) the nucleotide sequence set forth in SEQ ID NO: 29;
- (vi) the nucleotide sequence set forth in SEQ ID NO: 30;
- (vii) the nucleotide sequence set forth in SEQ ID NO: 31;
- (viii) the nucleotide sequence set forth in SEQ ID NO: 32;
- (ix) the nucleotide sequence set forth in SEQ ID NO: 33;
- (x) the nucleotide sequence set forth in SEQ ID NO: 34;
- (xi) a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of:
  - (a) KVGGLGDVVTs;
  - (b) GHTVEVILPKY;
  - (c) HDWSSAPVAWLYKEHY;
  - (d) GILNGIDPDIWDPYTD;
  - (e) DVPIVGIITRLTAQKG;
  - (f) NGQVLLGSA;
  - (g) AGSDFIIVPSIFPCGLTQLVAMRYGS;
  - (h) TGGLVDTV;
  - (i) KTGGLGDVAGA;
  - (j) GHRVMVVVPRY;
  - (k) NDWHTALLPVYLKAYY;
  - (l) GIVNGIDNMEWNPEVD;
  - (m) DVPLLGFGRLDGQKG;
  - (n) DVQLVMLGTG;
  - (o) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

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(p)VGG(V/L)RDTV;

- (xii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 37;
- (xiii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 38; and
- (xiv) a nucleotide sequence which is complementary to any one of (i) to (xiii).

12. An isolated or recombinant polypeptide, protein or enzyme comprising an amino acid sequence selected from the following:

- (i) the amino acid sequence set forth in SEQ ID NO: 2 or the mature protein region thereof;
- (ii) the amino acid sequence set forth in SEQ ID NO: 4 or the mature protein region thereof;
- (iii) the amino acid sequence set forth in SEQ ID NO: 6 or the mature protein region thereof;
- (iv) the amino acid sequence set forth in SEQ ID NO: 8 or the mature protein region thereof;
- (v) the amino acid sequence set forth in SEQ ID NO: 10 or the mature protein region thereof;
- (vi) a wheat starch synthase polypeptide having at least about 85% identity overall to any one of (i) to (v).

13. The isolated or recombinant polypeptide according to claim 12 further comprising one or more amino acid sequences selected from the group consisting of:

- (a) KVGGLGDVVT;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;

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- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- (f) NGQVLLGSA;
- (g)AGSDFIIVPSIFEPCGLTQLVAMRYGS;
- (h)TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- (m) DVPLLGFGRDLGQKG;
- (n) DVQLVMLGTG;
- (o)AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and
- (p)VGG(V/L)RDTV.

14. The isolated or recombinant polypeptide according to claim 13 wherein the wheat starch synthase polypeptide comprises at least three of said amino acid sequences selected from the group consisting of (a) to (h).
15. The isolated or recombinant polypeptide according to claim 13 wherein the wheat starch synthase polypeptide comprises at least six of said amino acid sequences selected from the group consisting of (i) to (p).
16. The isolated or recombinant polypeptide according to claim 12 encoding a wheat starch synthase II polypeptide.

17. The isolated or recombinant polypeptide according to claim 12 encoding a wheat starch synthase III polypeptide.
18. An isolated or recombinant starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:
  - (a) GHTVEVILPKY;
  - (b) HDWSSAPVAWLYKEHY;
  - (c) DVPIVGIITRLTAQKG;
  - (d) NGQVVLLGSA;
  - (e) AGSDFIIVPSIFEPCGLTQLVAMRYGS;
  - (f) TGGLVDTV;
  - (g) GIVNGIDNMEWNPEVD; and
  - (h) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT.
19. The isolated or recombinant polypeptide according to claim 16 consisting of a wheat starch synthase II polypeptide which comprises an amino acid sequence selected from the group consisting of:
  - (i) SEQ ID NO: 2;
  - (ii) SEQ ID NO: 4;
  - (iii) SEQ ID NO: 6; and
  - (iv) a homologue of any one of (i) to (iii) having at least about 85% identity thereto.
20. The isolated or recombinant polypeptide according to claim 17 consisting of a wheat starch synthase III polypeptide which comprises an amino acid sequence selected from the group consisting of:

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- (i) SEQ ID NO: 8;
  - (ii) SEQ ID NO: 10; and
  - (iii) a homologue of (i) or (ii) having at least about 85% identity thereto.
21. The isolated or recombinant polypeptide according to claim 12 substantially free of conspecific or non-specific proteins.
22. A method comprising:
- (i) hybridising single-stranded or double-stranded mRNA, cDNA or genomic DNA with a nucleotide sequence selected from the group consisting of:
    - (a) the nucleotide sequence according to any one of claims 1 to 9;
    - (b) a probe or primer derived from a nucleotide sequence according to subparagraph (a) and comprising at least about 15 contiguous nucleotides of said nucleotide sequence in length; and
  - (ii) detecting the hybridised mRNA, cDNA or genomic DNA using a detecting means.
23. The method according to claim 22 wherein the detecting means consists of a reporter molecule covalently attached to the probe or primer molecule.
24. The method according to claim 22 wherein the detecting means consists of a polymerase chain reaction.
25. The method according to claim 22 wherein the probe or primer comprises a nucleotide sequence selected from the group consisting of:
- (i) the nucleotide sequence set forth in SEQ ID NO: 25;



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- (ii) the nucleotide sequence set forth in SEQ ID NO: 26;
- (iii) the nucleotide sequence set forth in SEQ ID NO: 27;
- (iv) the nucleotide sequence set forth in SEQ ID NO: 28;
- (v) the nucleotide sequence set forth in SEQ ID NO: 29;
- (vi) the nucleotide sequence set forth in SEQ ID NO: 30;
- (vii) the nucleotide sequence set forth in SEQ ID NO: 31;
- (viii) the nucleotide sequence set forth in SEQ ID NO: 32;
- (ix) the nucleotide sequence set forth in SEQ ID NO: 33;
- (x) the nucleotide sequence set forth in SEQ ID NO: 34;
- (xi) a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of:

- (a) KVGGLGDVWTS;
- (b) GHTVEVILPKY;
- (c) HDWSSAPVAWLYKEHY;
- (d) GILNGIDPDIWDPYTD;
- (e) DVPIVGIITRLTAQKG;
- (f) NGQVLLGSA;
- (g) AGSDFIIVPSIFPCGLTQLVAMRYGS;
- (h) TGGLVDTV;
- (i) KTGGLGDVAGA;
- (j) GHRVMVVVPY;
- (k) NDWHTALLPVYLKAYY;
- (l) GIVNGIDNMEWNPEVD;
- (m) DVPLLGFGRLDGQKG;

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(n) DVQLVMLGTG;

(o) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT; and

(p) VGG(V/L)RDTV;

(xii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 37;

(xiii) a nucleotide sequence comprising at least about 15 contiguous nucleotides of an intron region of SEQ ID NO: 38; and

(xiv) a nucleotide sequence which is complementary to any one of (i) to (xiii).

26. A method of assaying for the presence or absence of a wheat starch synthase polypeptide in a plant or a plant extract or isolated nucleic acid sample, said method at least comprising performing the method according to any one of claims 22 to 25.

27. The method according to claim 26 further comprising preparing the plant extract or nucleic acid sample.

28. A method of marker-assisted breeding and/or selection of a plant at least comprising performing the method according to any one of claims 22 to 25.

29. The method according to claim 28 further comprising selecting a plant which expresses a desirable wheat starch synthase characteristic.

30. The method according to claim 28 further comprising crossing a plant which expresses a desirable wheat starch synthase characteristic to another plant.

31. The method according to claim 30 further comprising selecting progeny of the cross

which expresses a desirable wheat starch synthase characteristic.

32. A plant produced by the method according to any one of claims 28 to 31 wherein said plant expresses a wheat starch synthase polypeptide at a desired level detectable using said method.
33. A method of modifying the starch content and/or starch composition of one or more tissues or organs of a plant, comprising expressing in said plant a nucleic acid molecule for a time and under conditions sufficient for the enzyme activity of one or more starch synthase isoenzymes to be modified, wherein said nucleic acid molecule is selected from the group consisting of:
  - (i) the isolated nucleic acid molecule according to any one of claims 1 to 9;
  - (ii) a fragment of (i) which comprises a nucleotide sequence capable of being expressed to down-regulate the expression of an endogenous wheat starch synthase isoenzyme of said plant; and
  - (iii) a fragment of (i) which encodes a functional wheat starch synthase isoenzyme of said plant.
34. The method according to claim 33 wherein the fragment at sub-paragraph (ii) is an antisense molecule, ribozyme molecule, co-suppression molecule, or gene-targeting molecule.
35. The method according to claim 33 further comprising introducing the nucleic acid molecule to an isolated plant cell, tissue, organ, or organelle.
36. The method according to claim 35 further comprising regenerating an intact plant from the isolated plant cell, tissue, organ, or organelle carrying the introduced nucleic acid molecule.

37. The method according to claim 35 wherein the nucleic acid molecule is introduced to the plant cell, tissue, organ, or organelle by introgression.
38. The method according to claim 35 wherein the nucleic acid molecule is introduced to the plant cell, tissue, organ, or organelle by transformation means.
39. An isolated promoter sequence comprising a nucleotide sequence selected from the group consisting of:
- (i) nucleotides 1 to about 287 of SEQ ID NO: 11;
  - (ii) nucleotides 1 to about 1416 of SEQ ID NO: 37;
  - (iii) nucleotides 1 to about 973 of SEQ ID NO: 38;
  - (iv) a fragment of any one of (i) to (iii) capable of conferring expression on a heterologous gene in a monocotyledonous plant cell, tissue or organ; and
  - (v) a complementary nucleotide sequence to any one of (i) to (iv).
40. The isolated promoter sequence according to claim 39 that is operable in the endosperm.
41. A plant carrying the isolated nucleic acid molecule according to any one of claims 1 to 9 as an exogenous complement to its genome.
42. A progeny of the plant according to claim 41 wherein said progeny carries the introduced nucleic acid molecule.
43. A propagule of the plant according to claim 41 or 42 wherein said propagule carries the

introduced nucleic acid molecule present in said plant.

44. A gene construct or vector which comprises the isolated nucleic acid molecule according to any one of claims 1 to 9 and one or more origins of replication.
45. The gene construct according to claim 44 further comprising a promoter sequence in operable connection with said isolated nucleic acid molecule.
46. A gene construct or vector which comprises the probe or primer according to claim 10 or 11 and one or more origins of replication.
47. A modified starch derived from the plant according to claim 32 or 41 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
48. A modified starch derived from the progeny according to claim 42 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said progeny.
49. A modified starch derived from the propagule according to claim 43 wherein said starch is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said propagule.
50. A food product comprising the modified starch according to any one of claims 47 to 49.
51. The food product according to claim 50 consisting of flour or a flour-based food product.

52. The food product according to claim 50 or 51 selected from the group consisting of: flour-based sauce; leavened bread; unleavened bread; pasta, noodle; cereal; snack food; cake; and pastry.
53. Use of the modified starch according to any one of claims 47 to 49 in the preparation of a food product for consumption by an animal or human.
54. A modified protein derived from the plant according to claim 32 or 41 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said plant.
55. A modified protein derived from the progeny according to claim 42 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said progeny.
56. A modified protein derived from the propagule according to claim 43 wherein said protein is modified by virtue of the use of the isolated nucleic acid according to claim 1 to produce said propagule.
57. A non-food product comprising the modified protein according to any one of claims 54 to 56.
58. The non-food product according to claim 57 selected from the group consisting of: films; coatings; adhesives; building materials; and packaging materials.
59. Use of the modified protein according to any one of claims 54 to 56 in the preparation of a non-food product.



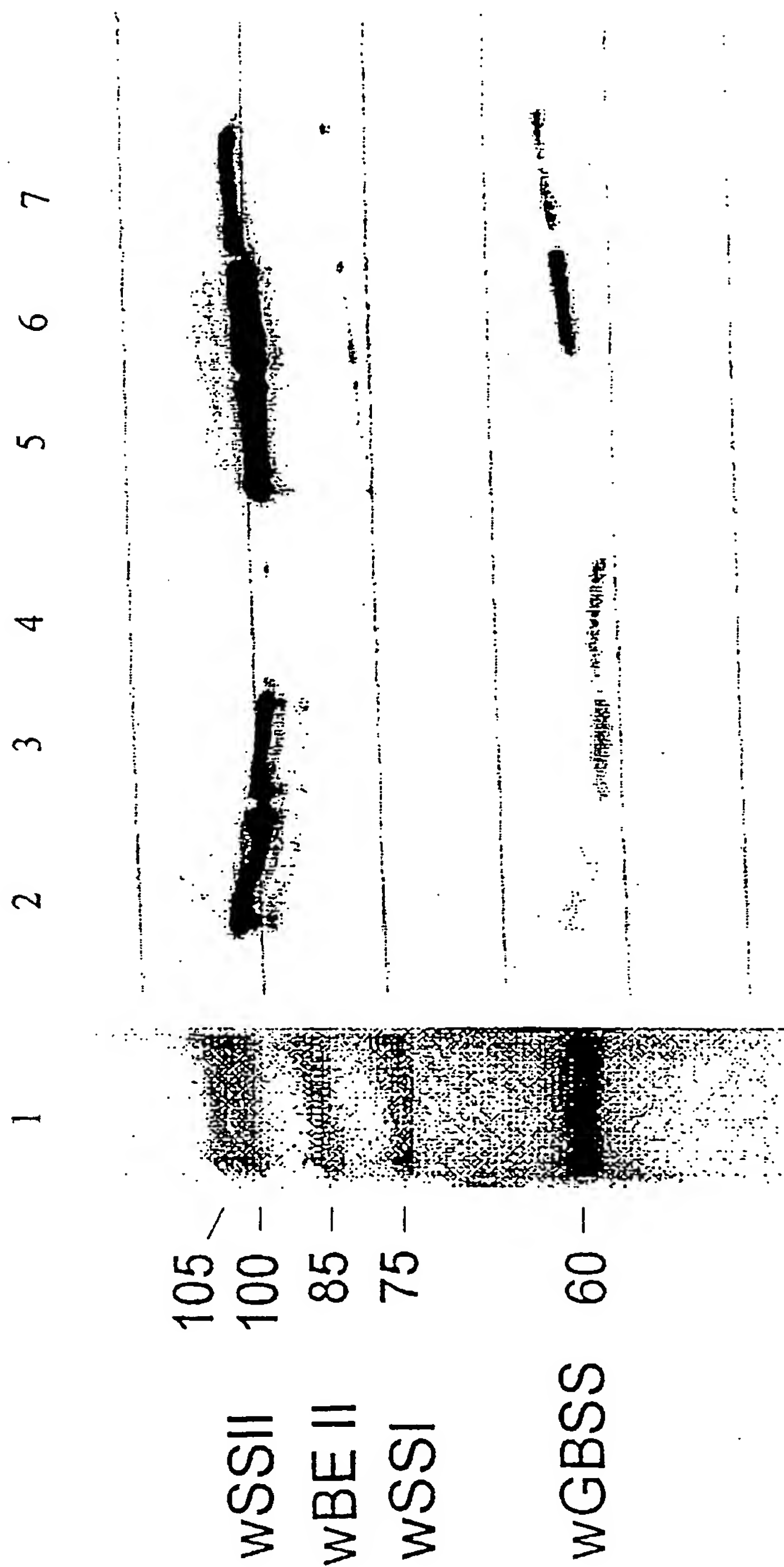


FIGURE 1

FIGURE 2A
FIGURE 2B
FIGURE 2C
FIGURE 2D
FIGURE 2E
FIGURE 2F
FIGURE 2G
FIGURE 2H
FIGURE 2I
FIGURE 2J
FIGURE 2K
FIGURE 2L
FIGURE 2M
FIGURE 2N
FIGURE 2O

FIGURE 2

1		50
WSSIIB	ATTCCCTCGG CCTGACCCCG TCGGTTTACC CCACACAGAG CACACTCCAG	
WSSIID	~~~~~	~~~~~
WSSIIA	~~~~~	~~~~~
51		100
WSSIIB	TCCAGTCCAG CCCACTGCCG CGCTACTCCC CACTCCCACT GCCACCACCT	
WSSIID	~~~~~	~~~~~
WSSIIA	~~~~~	~~~~~GCT GCCACCACCT
101		150
WSSIIB	CCGCcTGCGC CGCGCTCTGG GCGGACCAAC CCGCGCATCG TATCACGATC	
WSSIID	~~~~~	~~~~~
WSSIIA	CCGCCTGCGC CGCGCTCTGG GCGGAGGACC AACCCGCGCA TCGTACCATC	
151		200
WSSIIB	ACCCACCCCG ATCCCGGCCG CCGCCATGTC GTCGGCGGTC GCGTCCGCCG	
WSSIID	~~~~~	~~~~~
WSSIIA	GCCCGCCCCG ATCCCGGCCG CCGCCATGTC GTCGGCGGTC GCGTCCGCCG	

FIGURE 2A

201						250
WSSIIB	CGTCCTTCCT	CGCGCTCGCG	TCCGCCCTCCC	CCGGGAGATC	ACGGAGGAGG	
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
WSSIIA	CGTCCTTCCT	CGCGCTCGCC	TCCGCCCTCCC	CCGGGAGATC	ACGCAGGCGG	
251						300
WSSIIB	ACGAGGGTGA	GCGCGTCGCC	ACCCACACACC	GGGCTGGCA	GGTGCACTG	
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
WSSIIA	GCGAGGGTGA	GCGCGCCGCC	ACCCACGCC	GGGCCGGCA	GGTGCACTG	
301						350
WSSIIB	GCCGCCGTG	CCGCCGCAGC	GCACGGCTCG	CGACGGAGCG	GTGGCCCGCG	
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
WSSIIA	GCCGCCGTG	CCGCCGCAGC	GCACGGCTCG	CGACGGAGGT	GTGGCCCGCG	
351						400
WSSIIB	GCGCCGCCG	GAAGAAGGAC	GCGGGGAT..	CGACGACGC	CGCGCCCGCG	
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
WSSIIA	GCGCCGCCG	GAAGAAGGAC	GCGAGGGTCG	ACGACGACGC	CGCGTCCGCG	

FIGURE 2B

**FIGURE 2C**

601							650
WSSIIB	TAAAGACAGC	GGGCTGC	CCG	CACCCGCACG	CGCGCCCCAG	CCGTCGAGCC	
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
WSSIIA	CAAAGACAGC	GGGCTgc	CCG	CACCCGcACG	CGCGCCCCAT	cCGTCGAcCC	
651							700
WSSIIB	AGAACAGAGT	ACCGGTGAAT	GGTGAAACA	AAGCTAACGT	CGCCTCGCCG		
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~		
WSSIIA	AgAACAgAGT	ACCAGTGAAC	GGTGAAACA	AAGCTAACGT	CGCCTCGCCG		
701							750
WSSIIB	CCGACGAGCA	TAGCCGAGGT	CGCGGCTCCG	GATCCCCGCAG	CTACCATTC		
WSSIID	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~		
WSSIIA	CCGACGAGCA	TAGCCGAGGT	CGTGGCTCCG	GATCCGCAG	CTACCATTC		
751							800
WSSIIB	CATCAGTGAC	AAGCGGCCAG	AGTCCGTTGT	CCCAGCCGAG	AAGGcgcgc		
WSSIID	~~~~~	~~~~~	~~~~~	~CCAGCTGAG	AAGACGCCGC		
WSSIIA	CATCAGTGAC	AAGCGGCCG	AGTCCGTTGT	CCCAGCCGAG	AAGCCGCCG		

FIGURE 2D



801					850
wSSIIB	CGtCgtcCgg	CtCAAATtTc	gtgCcCtCgg	cttctGctCc	cggGtctGAC
wSSIID	CGTCGTCCGG	CTCAAATtTc	GAGTCCTCGG	CCTCTGCTCC	CGGGTCTGAC
wSSIIA	CGTCGTCCGG	CTCAAATtTc	GTGgTCTCGG	CTTCTGCTCC	CAGGCTGGAC
851					900
wSSIIB	actgtCaGCG	acGtGGaact	TgaActGAAG	aAGGGtgCgg	tCattgTcaA
wSSIID	ACTGTCAGCG	ACGTGGAACA	AGAACTGAAG	AAGGGTGCGG	TCGTTGTCGA
wSSIIA	ATTGACAGCG	ATGTTGAACC	TGAACTGAAG	AAGGGTGCGG	TCATCGTCGA
901					950
wSSIIB	aGAAgcTcCa	aaCcCaAaAG	CTCTTTTCGCC	GCCCGCAGCA	CCCGCTGTAC
wSSIID	AGAAGCTCCA	AAGCCAAAGG	CTCTTTTCGCC	GCCTGCAGCC	CCCGCTGTAC
wSSIIA	AGAAGCTCCA	AACCCAAAGG	CTCTTTTCGCC	GCCTGCAGCC	CCCGCTGTAC
951					1000
wSSIIB	AACAAGACCT	TTGGGACTTC	AAGAAATACA	TTGGTTTCGA	GGAGCCCCGTG
wSSIID	AAGAAGACCT	TTGGGAtTTC	AAGAAATACA	TTGGTTTCGA	GGAGCCCCGTG
wSSIIA	AAGAAGACCT	TTGGGACTTC	AAGAAATACA	TTGGCTTCGA	GGAGCCCCGTG

FIGURE 2E

1001	1050
wSSIIB	GAGGCCAAGG ATGATGGCCG GGCTGTTGCA GATGATGCGG GTCCTTCGA
wSSIID	GAGGCCAAGG ATGATGGCCG GGCTGTCGCA GATGATGCGG GTCCTTtGA
wSSIIA	GAGGCCAAGG ATGATGGCTG GGCTGTTGCA GATGATGCGG GTCCTTTGA
1051	1100
wSSIIB	ACACCAACCAG AATCACGATT CCGGCCCTTT GGCAGGGGAG AACGTCATGA
wSSIID	ACACCAACCAG AATCACGACT CCGGacCTTT GGCAGGGGAG AAtGTCATGA
wSSIIA	ACATCAACCAG AACCATGATT CCGGACCTTT GGCAGGGGAG AACGTCATGA
1101	1150
wSSIIB	ACGTGGTCGT CGTGGCTGCT GAATGTTCTC CCTGGTGCAA AACAGGTGGT
wSSIID	ACGTGGTCGT CGTGGCTGCT GAgTGTtCTC CCTGGTGCAA AACAGGTGGT
wSSIIA	ACGTGGTCGT CGTGGCTGCT GAATGTTCTC CCTGGTGCAA AACAGGTGGT
1151	1200
wSSIIB	CTTGGAGATG TTGCCGGTGC TTTGCCCAAG GCTTTGGCGA AGAGAGGACA
wSSIID	CTgGGAGATG TTGCgGGTGC TcTGCCCAAG GCTTTGGCaA AGAGAGGACA
wSSIIA	CTTGGAGATG TTGCCGGTGC TTTGCCCAAG GCTTTGGCGA AGAGAGGACA

FIGURE 2F

	1201				1250
WSSIIB	TCGTGTTATG	GTTGTGGTAC	CAAGGTATGG	GGA	CTATGAG
					GAAGCCTACG
WSSIID	TCGTGTTATG	GTTGTGGTAC	CAAGGTATGG	GGA	CTATGAA
					GAACCTACGg
WSSIIA	TCGTGTTATG	GTTGTGGTAC	CAAGGTATGG	GGA	CTATGAG
					GAAGCCTACG
	1251				1300
WSSIIB	ATGTCGGAGT	CCGAAAATAC	TACAAGGCTG	CTG	GACAGGA
					TATGGAAGTG
WSSIID	ATGTCGGAGT	CCGAAAATAC	TACAAGGCTG	CTG	GACAGGA
					TATGGAAGTG
WSSIIA	ATGTCGGAGT	CCGAAAATAC	TACAAGGCTG	CTG	GACAGGA
					TATGGAAGTG
	1301				1350
WSSIIB	AATTATTTC	ATGCTTATAT	CGATGGAGTT	GAT	TTGTGT
					TCATTGACGC
WSSIID	AATTATTTC	ATGCTTaTAT	CGATGGAGTT	GAT	TTGTGT
					TCATTGACGC
WSSIIA	AATTATTTC	ATGCTTATAT	CGATGGAGTT	GAT	TTGTGT
					TCATTGACGC
	1351				1400
WSSIIB	TCCTCTCTTC	CGACACCGCC	AGGAAGACAT	TTAT	GGGGC
					AGCAGACAGG
WSSIID	TCCTCTCTTC	CGACACCGAG	AGGAAGACAT	TTAT	GGGGC
					AGCAGACAGG
WSSIIA	TCCTCTCTTC	CGACACCGCC	AGGAAGACAT	TTAT	GGGGC
					AGCAGACAGG

FIGURE 2G

	1401				1450
wSSIIB	AAATTATGAA	GCGCATGATT	TTGTTCTGCA	AGGCCGCTGT	CGAGGTTCCA
wSSIID	AAATTATGAA	GCGCATGATT	TTGTTCTGCA	AGGCCGCTGT	TGAGGTTCCA
wSSIIA	AAATTATGAA	GCGCATGATT	TTGTTCTGCA	AGGCCGCTGT	CGAGGTTCCT
	1451				1500
wSSIIB	TGGCACGTTT	CATGCGGCGG	TGTCCCCTTAT	GGGGATGGAA	ATCTGGTGTT
wSSIID	TGGCACGTTT	CATGCGGCGG	TGTCCCCTTAT	GGGGATGGAA	ATCTGGTGTT
wSSIIA	TGGCACGTTT	CATGCGGCGG	TGTCCCCTTAT	GGGGATGGAA	ATCTGGTGTT
	1501				1550
wSSIIB	TATTGCAAAT	GATTGGCACA	CGGCACTCCT	GCCTGTCTAT	CTGAAAGCAT
wSSIID	TATTGCAAAT	GATTGGCACA	CGGCACTCCT	GCCTGTCTAT	CTGAAAGCAT
wSSIIA	TATTGCAAAT	GATTGGCACA	CGGCACTCCT	GCCTGTCTAT	CTGAAAGCAT
	1551				1600
wSSIIB	ATTACAGGGA	CCATGGTTTG	ATGCAGTACA	CTCGGTCCAT	TATGGTGATA
wSSIID	ATTACAGGGA	CCATGGTTTG	ATGCAGTACA	CTCGGTCCAT	TATGGTGATA
wSSIIA	ATTACAGGGA	CCATGGTTTG	ATGCAGTACA	CTCGGTCCAT	TATGGTGATA

FIGURE 2H

	1601						1650
WSSIIB	CATAACATCG	CTCACCAGGG	CCGTGGCCCA	GTAGATGAGT	TCCCCGTTAC		
WSSIID	CATAACATCG	CTCACCAGGG	CCGTGGCCCT	GTAGATGAAT	TCCCCGTTAC		
WSSIIA	CATAACATCG	CGCACCAGGG	CCGTGGCCCA	GTAGATGAAT	TCCCCGTTAC		
	1651						
WSSIIB	CGAGTTGCCT	GAGCACTACC	TGGAACACTT	CAGACTGTAC	GACCCCGTGG		
WSSIID	CGAGTTGCCT	GAGCACTACC	TGGAACACTT	CAGACTGTAC	GACCCCGTGG		
WSSIIA	CGAGTTGCCT	GAGCACTACC	TGGAACACTT	CAGACTGTAC	GACCCCGTGG		
	1701						
WSSIIB	GTGGTGAACA	CGCCAACTAC	TTCGCCGCCG	GCCTGAAGAT	GGCGGACCAG		
WSSIID	GTGGTGAACA	CGCCAACTAC	TTCGCCGCCG	GCCTGAAGAT	GGCGGACCAG		
WSSIIA	GTGGTGAGCA	CGCCAACTAC	TTCGCCGCCG	GCCTGAAGAT	GgCGGACCAG		
	1751						
WSSIIB	GTTGTCGTGG	TGAGCCCCGG	GTACCTGTGG	GAGCTGAAGA	CGGTGGAGGG		
WSSIID	GTTGTCGTGG	TGAGCCCCGG	GTACCTGTGG	GAGCTGAAGA	CGGTGGAGGG		
WSSIIA	GTTGTCGTGG	TGAGCCCCGG	GTACCTGTGG	gAGCTCAAGA	CGGTGGAagg		

FIGURE 2I

	1801				1850
WSSIIB	CGGCTGGGGG	CTTCACGACA	TCATACGGCA	GAACGACTGG	AAGACCCGCG
WSSIID	CGGCTGGGGG	CTTCACGACA	TCATACGGCA	GAACGACTGG	AAGACCCGCG
WSSIIA	CGGCTGGGGG	CTTCACGACA	TCATACGGCA	GAACGACTGG	AAGACCCGCG
	1851				1900
WSSIIB	GCATCGTGAA	CGGCATCGAC	AACATGGAGT	GGAACCCCGA	GGTGGACGTC
WSSIID	GCATCGTCAA	CGGCATCGAC	AACATGGAGT	GGAACCCCGA	GGTGGACGCC
WSSIIA	GCATCGTCAA	CGGCATCGAC	AACATGGAGT	GGAACCCCGA	GGTGGACGTC
	1901				1950
WSSIIB	CACCTCAAGT	CGGACGGCTA	CACCAACTTC	TCCCTGGGGA	CGCTGGACTC
WSSIID	CACCTCAAGT	CGGACGGCTA	CACCAACTTC	TCCCTGAGGA	CGCTGGACTC
WSSIIA	CACCTCAAGT	CGGACGGCTA	CACCAACTTC	TCCCTGGGGA	CGCTGGACTC
	1951				2000
WSSIIB	CGGCAAGCGG	CAGTGCAAGG	AGGCCCTGCA	GCGGGAGCTG	GGCCTGCAGG
WSSIID	CGGCAAGCGG	CAGTGCAAGG	AGGCCCTGCA	GCGCGAGCTG	GGCCTGCAGG
WSSIIA	CGGCAAGCGG	CAGTGCAAGG	AGGCCCTGCA	GCGCGAGCTG	GGCCTGCAGG

FIGURE 2J



2001	2050
wSSIIIB	TCCGCGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
wSSIID	TCCGCGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
wSSIIA	TCCGCGGCGA CGTGCCGCTG CTCGGCTTCA TCGGGCGCCT GGACGGGCAG
2051	2100
wSSIIIB	AAGGGCGTGG AGATCATCGC GGACGCGATG CCCTGGATCG TGAGCCAGGA
wSSIID	AAGGGCGTGG AGATCATCGC GGACGCCATG CCCTGGATCG TGAGCCAGGA
wSSIIA	AAGGGCGTGG AGATCATCGC GGACGCCATG CCCTGGATCG TGAGCCAGGA
2101	2150
wSSIIIB	CGTGCAGCTG GTCATGCTGG GCACCGGGCG CCACGACCTG GAGGCATGC
wSSIID	CGTGCAGCTG GTGATGCTGG GCACCGGGCG CCACGACCTG GAGGCATGC
wSSIIA	CGTGCAGCTG GTCATGCTGG GCACCGGGCG CCACGACCTG GAGGCATGC
2151	2200
wSSIIIB	TGCGGCACTT CGAGCGGGAG CACCACGACA AGGTGCGCGG GTGGGTGGG
wSSIID	TGCGGCACTT CGAGCGGGAG CACCACGACA AGGTGCGCGG GTGGGTGGG
wSSIIA	TGCGGCACTT CGAGCGGGAG CACCACGACA AGGTGCGCGG GTGGGTGGG

**FIGURE 2K**

2201				2250	
WSSIIB	TTCTCCGTGC	GGCTGGCGCA	CCGGATCACG	GCCGGCGCCG	ACGCGCTCCT
WSSIID	TTCTCCGTGC	GCCTGGCGCA	CCGGATCACG	GCGGGGGCGG	ACGCGCTCCT
WSSIIA	TTCTCCGTgc	GccTGGCGCA	CCGGATCACG	GCGGGCGCCG	ACGCGCTCct
2251				2300	
WSSIIB	CATGCCCTCC	CGGTTCGAGC	CGTGCGGACT	GAACCAGCTC	TACGCCATGG
WSSIID	CATGCCCTCC	CGGTTCGTGC	CGTGCGGGCT	GAACCAGCTC	TACGCCATGG
WSSIIA	CATGCCCTCC	CGGTTCGAgc	CGTGCGGGTt	GAACCAGCTt	TACGCCATGG
2301				2350	
WSSIIB	CCTACGGCAC	CGTCCCCGTC	GTGCATGCCG	TCGGTGGCCT	GAGGGACACC
WSSIID	CCTACGGCAC	CGTCCCCGTC	GTGCACGCCG	TCGGCGGCCT	CAGGGACACC
WSSIIA	CCTACGGCAC	CGTCCCCGTC	GTGCACGCCG	TCGGCGGGGT	GAGGGACACC
2351				2400	
WSSIIB	GTGCCGCCGT	TCGACCCCTT	CAACCACTCC	GGGCTCGGGT	GGACGTTCGA
WSSIID	GTGCCGCCGT	TCGACCCCTT	CAACCACTCC	GGGCTCGGGT	GGACGTTCGA
WSSIIA	GTGCCGCCGT	TCGACCCCTT	CAACCACTCC	GGcCTCGGGT	GGACGTTCGA

FIGURE 2L

	2401					2450
wSSIIB	CCGCGCAGAG	GCGCAGAAGC	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	
wSSIID	CCGCGCCGAG	GCGCACAAAGC	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	
wSSIIA	CCGCGCCGAG	GCGCACAAAGC	TGATCGAGGC	GCTCGGGCAC	TGCCTCCGCA	
	2451					2500
wSSIIB	CCTACCGGGA	CTACAAGGAG	AGCTGGAGGG	GGCTCCAGGA	GCGCGGCATG	
wSSIID	CCTACCGGAG	CTTCAAGGAG	AGCTGGAGGG	CCCTCCAGGA	GCGCGGCATG	
wSSIIA	CCTACCGGGA	CTACAAGGAG	AGCTGGAGGG	GcCTCCAGGA	GCGCGGCATG	
	2501					2550
wSSIIB	TCGCAGGACT	TCAGCTGGGA	GCATGCCGCC	AAGCTCTACG	AGGACGTCCT	
wSSIID	TCGCAGGACT	TCAGCTGGGA	GCACGCCGCC	AAGCTCTACG	AGGACGTCCT	
wSSIIA	TCGCAGGACT	TCAGCTGGGA	GCATGCCGCC	AAGCTCTACG	AGGACGTCCT	
	2551					2600
wSSIIB	CGTCAAGGCC	AAGTACCAGT	GGTGAACGCT	AGCTGCTAGC	CGGTCCAGCC	
wSSIID	CGTCAAGGCC	AAGTACCAGT	GGTGAACGCT	AGCTGCTAGC	CGGTCCAGCC	
wSSIIA	CcTCAAGGCC	AAGTACCAGT	GGTGAACGCT	AGCTGCTAGC	CGGTCCAGCC	

FIGURE 2M

## FIGURE 2N

2801	GGAATGTTGT	TAACTTGGTA	TTGTAATTG	TTATGTTGTG	TGCATTATTA	2850
WSSIIB						
2802	TTGTTATGTT	GTGTGCATTA	TTACAATGTT	GTTACTTATT	CTTGTTAAGT	
WSSIID						
2803	GGAATGTTGT	CAACTTGGTA	TTGTAgTTTG	CTATGTTGTa	TGCgTTATTA	
WSSIIA						
2851	CAGAGGGCAA	CGATCTGCGC	CGGCGCACCG	GCCCAACTGT	TGGGCCGGTC	2900
WSSIIB						
2852	CGGAGGCCAA	GGGCGAAAGC	TAGCTCACAT	GTCTGATGGA	TGCAAAAAAA	
WSSIID						
2853	caatgttggt	acttattcct	gtTAAAAAAA	AAAAA~~~~~		
WSSIIA						
2901	GCACAGCAGC	CGTTGGATCC	GACCGCCTGG	GCCGTTGGAT	CCCACCGAAA	2950
WSSIIB						
2902	AAAAA~~~~~	AAA~~~~~	~~~~~	~~~~~	~~~~~	
WSSIID						
2903	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
WSSIIA						
2951	AAAAA~~~~~	AAAAA				2965
WSSIIB						
2952	~~~~~	~~~~~				
WSSIID						
2953	~~~~~	~~~~~				
WSSIIA						

FIGURE 20

FIGURE 3A
FIGURE 3B
FIGURE 3C
FIGURE 3D
FIGURE 3E
FIGURE 3F
FIGURE 3G

FIGURE 3



WSSIIA	1	MSSAVASAAS	---	FLALASA	SP-GRSRRRA	RVSAPPPHAG	AGRL----	HW	PPWPP-QRTA	51
WSSIIB	1	*****	---	*****	**--*****T	***S***T*	***-----**		**S**--***	51
WSSIID		-----	-----	-----	-----	-----	-----		-----	
ZSSIIA	1	***AV*SS*	STF*****	**G**--**	**GSS*F*T*	*-S*SFAFWA			**S**RAPRD	57
ZSSIIB	1	*PG*-I*SS*	SAFL*PV**S	**--R***G	S*G*ALRSY*	YSGAELRL**			ARRG*P*DG*	56
PEASSII	1	*MLSLG*D*T	VLP*H*KNLK	FTP*KL*TLNG	--DLAFSKGL	GVGRNLNCGSV			-----R	49
POTSSII	10	PVNFIFCDFY	VMENSI*LHS	GNQFHPNLPL	---LALRPKK	LSLIHGSSRE			-----Q	57
↓ Transit peptide cleavage site										
WSSIIA.	52	RDGGVAARAA	GKKDARVDDD	AASARQPRAR	RGGAATKVAE	RRDPVKTLDR			DAAEGGAPAP	111
WSSIIB	52	***A*****	*****GI--**	**P*****L	*****	*****			*****S*	110
WSSIID		-----	-----	-----	-----	-----			-----	
ZSSIIA	58	AALVR*EAE*	*G**PPERS	GDA**L***	*---NA*SK	***			-----	97
ZSSIIB	57	-ASVR**A*P	AGG-----	-----	-----	-----			-----	68
PEASSII	50	LNHKQHV**V	**SFGADENG	DG*EDDVVNA	TIEKSK**LA	LQRELIQQIA			ERKKLVSSID	109
POTSSII	58	MWRNQRVK*T	*ENSGEAA-S	*DESDALQV	TIEKSK**LA	MQQDLLQQIA			ERRKVVSSIK	116

FIGURE 3A

20/50

WSSIIA	112	PAPRQDAARP	PSMNGTPVNG	ENKSTGGGGA	TKDSGLPAPA	RAPHPSTQNR	VPVNGENKAN	171
WSSIIB	111	***ED**L	***M***	*****	*****	***Q**S**	*****	170
WSSIID		-----	-----	-----	-----	-----	-----	
ZSSIIA	98	-----	-----	----LQPVG	RYG*ATGNT*	*TGAA*C**A	ALADV*I*SI	132
ZSSIIB	69	-----	-----	-----	-ESEEAAKSS	SSSQAGAVQG	STAKAVDS*S	97
PEASSII	110	SDSIPGLEGN	GVSYESSEKS	LSR-----	-----	-----DS*P	QKGSSSSGSA	146
POTSSII	117	S-----SL*NA	KGTYDGGSGS	LSDVDIPDVD	KDYNVTVPST	A*TGITDVK	NTPPAISHDF	172

WSSIIA	172	VASPPTSIAE	VVAPDSAATI	SISDKAPESV	VPAEKPPSS	GSNFVVSASA	PRLDIDSDVE	231
WSSIIB	171	*****	*A***p***	*****	*****A***	*****P***	*GS*TV***	230
WSSIID	203	-----	-----	-----	*****T***	*****ES***	*GS*TV***	231
ZSSIIA	134	*A*****VK	FP**GYRMIL	PSG*I**T*	L**P**--LH	E*PA*DGD*N	--GIAPPT**	188
ZSSIIB	99	PPN*L**AFK	QSQAAMQNG	TSGSSASTA	A*VSG*KADH	P*AP*TKREI	DASAVKPEPA	158
PEASSII	147	*ETKR--WHC	FQQ-----LC	RSKETETWA*	SSVGINQGF	EIEKKND*VK	ASSKLHFNEQ	199
POTSSII	173	*E*KREIKRD	LADERAPPLS	RS*IT*SSQI	SSTVSSK--R	TL*VPETPK	SSQETLL**N	230

FIGURE 3B

wSSIIP1 Region									
WSSIIA	232	PELKKGAVIV	EEAPNPKALS	PPAAPAVQED	LWDFKKYIGF	EEPVEAKDDG	WAVADDAGSF		291
WSSIIB	231	L*****	K*****	*****Q*	*****	*****	R*****		290
WSSIID	232	Q*****V*	*****K*****	*****	*****	*****	R*****		291
ZSSIIA	189	*-----	-----	-----L**A	T*****	D**D*****S	RVG*****		224
ZSSIIB	159	GDDARPVESI	-----	-----	-----*I	A**D**A*-	A*P*T**AAS		188
PEASSII	200	IKN*LYERPD	TKDIS--SSI	R-----	-----TSSL	KFENFEGANE	PSSKEV*NEA		242
POTSSII	231	SRKSLVD*PG	KKIQSYMPSL	R-----	-----*ESSAS	HVEQRNENLE	GSS*EANEET		277

Region 1									
WSSIIA.	292	EHHQNH--S	GPLAGENVMN	VVVVAAECSP	WCKTGGLGDV	AGALPKALAK	RGHRVMVVVP	Region 2	349
WSSIIB	291	*****--*	*****	*****	*****	*****	*****		348
WSSIID	292	*****--*	*****	*****	*****	*****	*****		349
ZSSIIA	225	**YGDN*--*	*****	I*****	V*****	V*****R	*****		282
ZSSIIB	189	APYDRE*NEP	*****P*****	*****S**A*	F*****	V*****R	*****I*		248
PEASSII	243	*NFESGGEKP	P***T***	IIL*S**A*	*S*****	*S*****R	*****I*A*		302
POTSSII	278	*DPV*I*EKP	P***T***	IIL*S**A*	*S*****	*S*****R	*****A*		337

FIGURE 3C

Sgp-1 Peptide 3									
WSSIIA	350	RYGDYEEAYD	VGVRKYKAA	GQDMEVNYEH	AYIDGVDFVF	IDAPLFRHRQ	EDIYGGSRQE	409	
WSSIIB	349	*****	*****	*****	*****	*****	*****	408	
WSSIID	350	*****PT*	*****	*****	*****	*****E	*****	409	
ZSSIIA	283	*****V**F*	**I*****	**L*****	*F*****	*****	*****	342	
ZSSIIB	249	**E**A**R*	L**RR**V*	**S**T***	S*****	VE**P***H	NN***E*LD	308	
PEASSII	303	H**N**A**H*	I***R**V*	*****T***	T*****I**	**S*I**NLE	SN***N*LD	362	
POTSSII	338	**DN*P*PQ*	S***I**VD	***VD*T**Q	*LLMDC****	*HSHM***IG	NN***N*VD	397	

Region 3									
WSSIIA	410	IMKRMILFCK	AAVEVPWHVP	CGGVPYGDGN	LVFIANDWHT	ALLPVYLKAY	YRDHGLMQYT	469	
WSSIIB	409	*****	*****	*****	*****	*****	*****	468	
WSSIID	410	*****	*****	*****	*****	*****	*****	469	
ZSSIIA	343	*****	V*****	***C*****	*****	*****	*****	402	
ZSSIIB	309	*L*****	*****YA*	**TV*****	*****	*****	*****	368	
PEASSII	363	*LR**V*****	*****	**IC*****	*****	*****	*****A	422	
POTSSII	398	*L***V*****	**I*****	***C*****	*****	***A*****	***N*I*N**	457	

FIGURE 3D

WSSIIA	470	RSIMVIHNIA	HQGRGPVDEF	PFTLPPEHYL	EHFRLYDPVG	GEHANYFAAG	LKMADQVVVV	529
WSSIIB	469	*****	*****	*****	*****	*****	*****	528
WSSIID	470	*****	*****	*****	*****	*****	*****	529
ZSSIIA	404	*VL*****	*****	*YMD*****	Q**E*****	*****I*****	*****R**T*	462
ZSSIIB	369	*VL*****	*****D*	VNFD*****	D**K**NI*	*D*S*V*****	**T**R**T*	428
PEASSII	423	*VL*****	*****ED*	NTVD*SGN**	DL*KM*****	***F*I*****	**T**RI**T*	482
POTSSII	458	*VL*****	*****LED*	SYVD**P**M	DP*K*****	***F*I*****	**T**R**T*	517

Region 4

WSSIIA	530	SPGYLWELKT	VEGGWGLHDI	IRQNDWKTRG	IVNGIDNMEW	NPEVDVHLK-	SDGYTNFSLG	588
WSSIIB	529	*****	*****	*****	*****	*****	*****	587
WSSIID	530	*****	*****	*****	*****	*****A***-	*****R	588
ZSSIIA	463	*R*****	*****	*S*****IN*	*****HQ**	***K*****R-	*****Y**E	521
ZSSIIB	429	*N**M*****	S*****	*N*****LQ*	*****MS**	**A*****H-	**D***YTFE	487
PEASSII	483	*H**A*****	S*****N*	*NES***F**	***V*TKD*	**QF*AY*T-	*****YN*K	541
POTSSII	518	*H**S*****	SQ*****Q*	*NE***LQ*	*****TK**	***L*****PR	***M**Y**D	577

FIGURE 3E

Region 6									
	649	GTGRHDLESM	LRHFEREHHD	KVRGWGFSV	RLAHRITAGA	DALLMPSRFE	PCGLNQLYAM		
WSSIIA	649	*****G*	*****	*****	*****	*****	*****	708	
WSSIIB	648	*****	*****	*****	*****	*****	*****	707	
WSSIID	649	*****	*Q*****	*****	*****	*****	*****	708	
ZSSIIA	582	***A***R*	*Q*L*****PN	*****	PM*****	*V*****V	*****	641	
ZSSIIB	548	***A***D*	**R**S**S*	**A*****	P*****	*I*****	*****	607	
PEASSII	602	***A***Q*	*KE**AQ*C*	*I*S*****	KM*****S	*I*****	*****	661	
POTSSII	638	***R***Q*	**Q**CQ*N*	*I*****	KTS*****	*I*****	*A*****	697	

## FIGURE 3F



Region 7									
WSSIIA	709	AYGTVPVVHA	VGVRDTPVP	FDPFNHSLG	WTFDRAEAK	LIEALGHCLR	TYRDYKESWR		768
WSSIIB	708	*****	***L*****	*****	*****Q*	*****	*****		767
WSSIID	709	*****	***L*****	*****	*****	*****	***F*****		768
ZSSIIA	642	*****	***L*****A*	***GDA***	*****N*	***R***D	***K*G***K		701
ZSSIIB	608	*****	***L*****A*	***DT***	*****NR	M*D*S***T	***N*****		667
PEASSII	662	*****G	***L*****Q*	*N*DE**V*	*****N*	*MA**WN**L	***K***K**E		721
POTSSII	698	K***I*****	***L*****Q*	***LMSQDW*	GPS*****SQ	**PRIRN**L	***E***K**E		757

WSSIIA	769	GLQERGMSQD	FSWEHAAKLY	EDVLLKAKYQ	W	799
WSSIIB	768	*****	*****	***V*****	*	798
WSSIID	769	*****	*****	***V*****	*	799
ZSSIIA	702	S**A*****	L**D***E**	***V*****	*	732
ZSSIIB	668	ACRA**AE*	L**D***V**	***V*****	*	698
PEASSII	722	*I*****	L**DN**QQ*	*E**VA*****	*	752
POTSSII	759	*I*T*C*T**	L**DN**QN*	*E**IA*****	*	788

FIGURE 3G

26/50

Pre-  
leaf  
anthesis

4 6 8 10 12 15 18 21 25



**FIGURE 4**

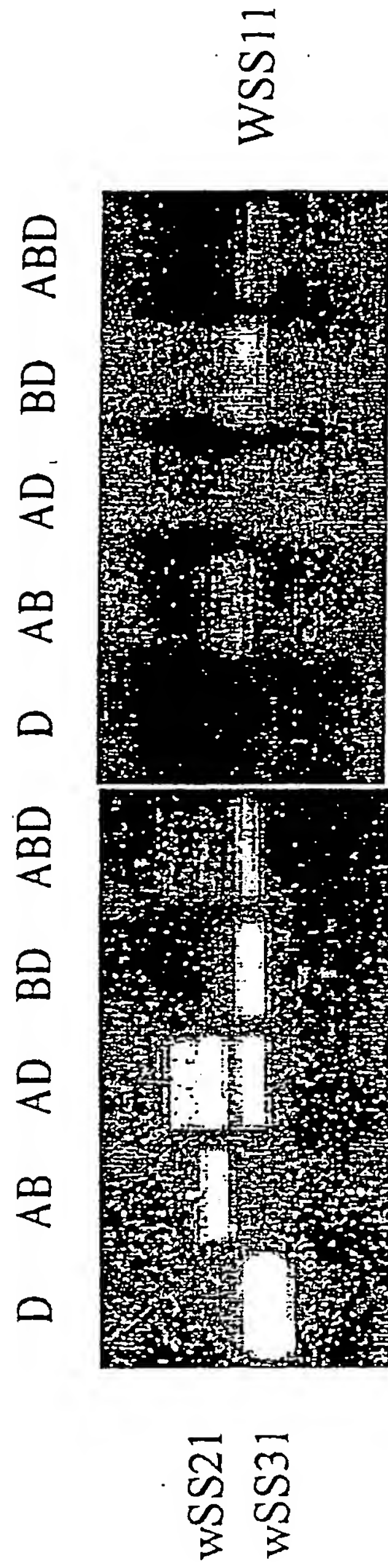
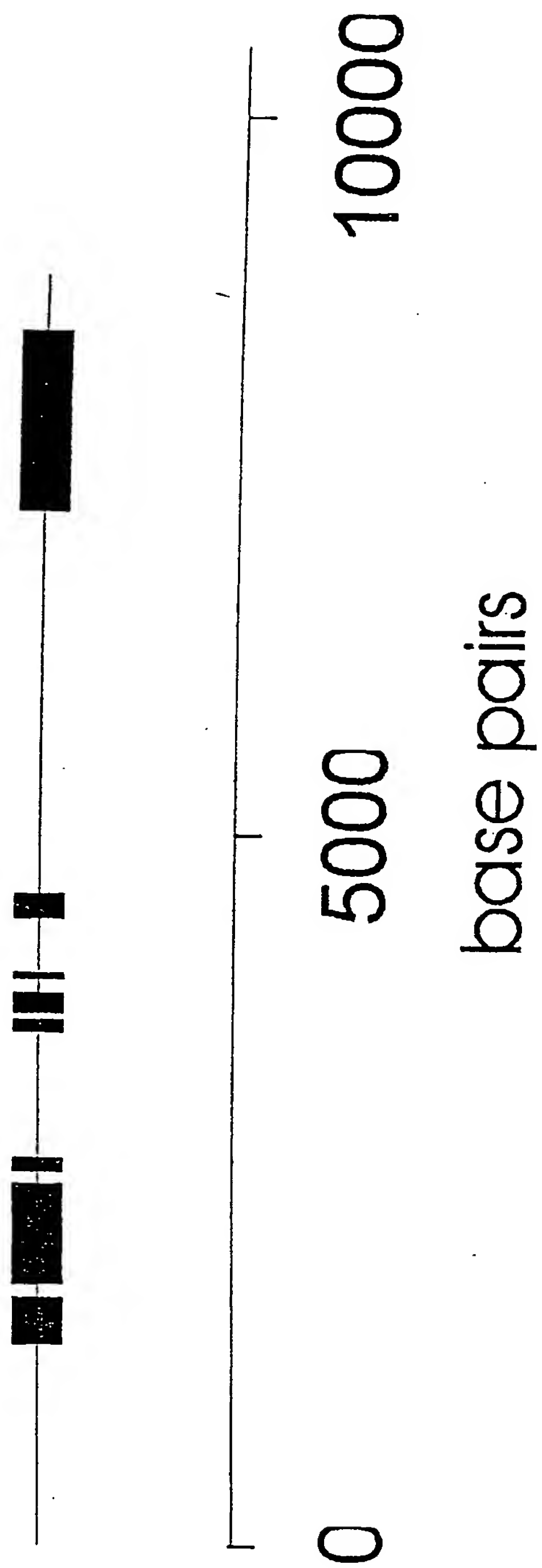


FIGURE 5



## FIGURE 6

FIGURE 7A
FIGURE 7B
FIGURE 7C
FIGURE 7D
FIGURE 7E
FIGURE 7F
FIGURE 7G
FIGURE 7H
FIGURE 7I

**FIGURE 7**

1	MEMSLWPRSP	LCPRSRQPLV	VVRP..AGRG	GLTQPFLMNG	RFTRSRTRLRC	50
wSSIII	MEMVLRSP	LCLRS.GPVL	IFRPTVAGGG	GGTQSLRLTT	RFARRRVIRC	
mSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
51	MVASSDPPNR	KSRMVPPQV	KVISSRGYTT	RLIVEPSNEN	TEHNNRD...	100
wSSIII	VVASPGCPNR	KS.RTASPNV	KVAAYSNYAP	RLLVESSEKK	SEHHDSSRHR	
mSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
101	EETLDTYNAL	LSTETAEWTD	NREAE.....	..TAKADSSQ	NALSSSIIGE	150
wSSIII	EETIDTYNGL	SGSDAAELTS	NRDVEIEVDL	QHISEEELPG	KVSINASLGE	
mSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
151	VDVAD.....	EDILAADLTV	YSLSSVMKKE	VDAADKARVK	EDAFELDLPA	200
wSSIII	METVDEAEVE	EDKFEVDTS	IVLRNVAVRE	VDPKDEHNAK	.DVFVVDSSG	
mSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	

FIGURE 7A



	201			250
wSSIII	TTLRSVIVDV	MDHNGTVQET	LRSVIVDVMD	.HNGTVQE.. TLRSVIVDVM
mSSIII	TAPDNAAVEE	VVDEAEVEED	MVDVDILGLD	LNNATIEEID LMEEALLENF
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~
	251			300
wSSIII	D.DAADKARV	EEDVFELDLS	GNISSAT..	.....TVEL
mSSIII	DVDSPGNASS	GRTYGGVDEL	GELPSTSVDC	IAINGKRRSL KPKPLPIVRF
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~
	301			350
wSSIII	DAVDEVGPVQ	DKFEATSSGN	VSNSATVREV	DASDE...AG NDQGIFRADL
mSSIII	QEQQIVLSI	VDEEGLIASS	CEEGQPVVDY	DKQEEENSTAF DEQKQLTDDF
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~
	351			400
wSSIII	SGNVFSSSTT	VEVG..AVDE	AGSIKDRFET	DSSGNVSTSA PMWDAIDETV
mSSIII	PEEGISIVHF	PEPNNDIVGS	SKFLEQKQEL	DGSYKQDRST TGLHEQDQSV
pSSIII	~~~~~	~~~~~	~~~~~	~~~~~

FIGURE 7B

	401		450
wSSIII	ADQDTFEADL	SGNASSCATY	REVDDVVDET RSEETFAMD LFASESGHEK
mSSIII	VSSHGQDKSI	VG.VPQQIQY	NDQSIAGSHR QDQSIAGAPE QIQSVAGYIK
pSSIII	~~~~~	~~~~~	~~~~~MDVPPF
	451		500
wSSIII	HMAVDYVGEA	TDEEETYQQQ	YPVPSSFMSW DKAIKTGVS LNPELRLVRV
mSSIII	PNQ.SIVGSC	KQHELIIEP	KKIESIISYN EIDQSIVGSH KQDKSVVSV
pSSIII	PLHRSLSCTS	VSNAITHLKI	KPILGFVSHG TTSLSVQSSS WRKDGMTGV
	501		550
wSSIII	EEQGKVNFS	KKDLSIDDL	P GQNQSIIGSY KQDKSIADVA GPTQSI FGSS
mSSIII	EQIQSIVSHS	KPNQSTVDSY	RQAESIIGVP EKVQSI TSYD KLDQSI VLSL
pSSIII	SFSICANFSG	RRRRKVSTPR	SQGSSPKGFV PRKPSGMSTQ RKVQKSNGDK
	551		600
wSSIII	KQHRSI VAFP	KQNQSI VSVT	EQKQSI VGFR SQDLSAVSL. ....P
mSSIII	KQDEPIISVP	EKIQSI VHYT	KPNQSI VGLP KQQQSI VHIV EPKQSI DGFP
pSSIII	ESKSTSTSKE	SEISNQKTVE	ARVETSDDDT KGVVRD HKFL EDEDEINGST

FIGURE 7C

801					850
wSSIII	ADSVIDLVLN	RDLTALANEP	DVVIKGAENG	WKWRLFTEKL	HKSDLGGVWW
mSSIII	ADSTIDLVLN	RDLTALANEP	DVVIKGAENG	WKWRLFTEKL	HKSELAGDWW
pSSIII	PDEDVEIFLN	RGLSTLNES	DVLIKGAENG	WKWRLFTEKL	TETHLNGDWW
851					900
wSSIII	SCKLYIPKEA	YRLDFVFFNG	RTVYENNGNN	DFCIGIEGTM	NEDLFEDFLV
mSSIII	CCKLYIPKQA	YRMDFVFFNG	HTVYENNNNN	DFVIQIESTM	DENLFEDFLA
pSSIII	SCKIHVPKEA	YRADFVFFNG	QDVYDNNNDGN	DFSITVKGGM	QIIDFENFLL
901					950
wSSIII	KEKQRELEKL	AMEEAERRTQ	TEEQRRRKEA	RAADEAVRAQ	AKAEIEIKKK
mSSIII	EKQRELENL	ANEEAERRRQ	TDEQRRMEEE	RAADKADRVQ	AKVEVETKKN
pSSIII	EKQRELEKL	AKEQAERERL	AEQRRRIEAE	KAEIEADRAQ	AKEEAAKKKK
951					1000
wSSIII	KLQSMLSLAR	TCVDNLWYIE	ASTDTRGDTI	RLYNNRNSRP	LAHSTEIWMH
mSSIII	KLCNVGLGLAR	APVDNLWYIE	PITGQEATV	RLYNNRNSRP	LHSTEIWMH
pSSIII	VLRELMVKAT	KTRDITWYIE	PSEFKCEDKV	RLYNNKSSGP	LSHAKDLWIH

FIGURE 7E

601	650
wSSIII KQ.NVPIVGT SREGQTKQVP VVDRQDALYV NGLEAKEGDH TSEKTEDDAL	
mSSIII KQ.DLSIVGI SNEFQTKQLA TVGTHDGLLM KGVEAKE... TSQKTEGDTL	
pSSIII KSISMSPVRV SSQFVESEET GGDDKDAVKL N..KSKRSEE SGFIIDSVIR	
651	700
wSSIII HVKFNVDNVL RKHQADRTQA VEKKTWKKVD EEHLYMTEHQ KRAA..EGQM	
mSSIII QATFNVDNLS QKQEGLTKEA DEITIEKIN DEDLVMIEEQ KSIAMNEEQT	
pSSIII EQSGSQGETN ASSKGSHAVG TKLYEILQVD VEPQQLKEN. .NAGNVEYKG	
701	750
wSSIII VVNEDELSIT EIGMGRGD.K IQHVLSEEL SWSEDEVQLI EDDGQYEVDE	
mSSIII IVTEEDIPMA KVEIGIDKAK FLHLLSEES SWDENEVGII EADEQYEVDE	
pSSIII PVASKLLEIT KA.....SD VEHTESNEID DLDN..SFF KSDLIEEDEP	
751	800
wSSIII TSVSVNVEQD IQGSPQDVVD PQALKVMLQE LAEKNYSMRN KLFVFPEVVK	
mSSIII TSMS..TEQD IQESPNDDDL PQALWSMLQE LAEKNYSLGN KLFTYPDVLK	
pSSIII LAAGTVETGD SSLNLRLEME ANLRRQAIER LAEENLLQGI RLFCFPEVVK	

FIGURE 7D

	1001				1050
wSSIII	GGYNNWTDGL	SIVESFVKCN	DKDGDWYAD	VIPPEKALVL	DWVFADGPAG
mSSIII	GGYNNWIDGL	SFAERLVHHH	DKDCDWWFAD	VVVPERTYVL	DWVFADGPPG
pSSIII	GGYNNWKDGL	SIVKKLVKSE	RIDGDWWYTE	VVIPDQALFL	DWVFADGPPK
	1051				1100
wSSIII	NARNYDNNAR	QDFHAILPNN	NVTEEGFWAQ	EEQNIYTRLL	QERREKEETM
mSSIII	SARNYDNNNGG	HDFHATLP.N	NMTEEEYWME	EEQRIYTRLQ	QERREREEAI
pSSIII	HAIAYDNNHR	QDFHAIVP.N	HIPEELYWVE	EEHQIFKTLQ	EERRLREAAM
	1101				1150
wSSIII	KRKAERSANI	KAEMKAKTMR	RFLSQKHIV	YTEPLEIRAG	TTVDVLYNPS
mSSIII	KRKAERNAKM	KAEMKEKTMR	MFLVSQKHIV	YTEPLEIHAG	TTIDVLYNPS
pSSIII	RAKVEKTALL	KTETKERTMK	SFLSQKHVV	YTEPLDIQAG	SSVTVYYNPA
	1151				1200
wSSIII	NTVLNGKSEG	WERCSENLWM	HSSGALPPQK	MVKSGDGPLL	KATVDVPPDA
mSSIII	NTVLTKKPEV	WERCSENRWM	YPGGVLPPQK	MVQAENGSHL	KATVYVPRDA
pSSIII	NTVLNGKPEI	WERCSENRWT	HRGLPLPPQK	MSPAENGTHV	RATVKVPLDA

FIGURE 7F

## FIGURE 7G



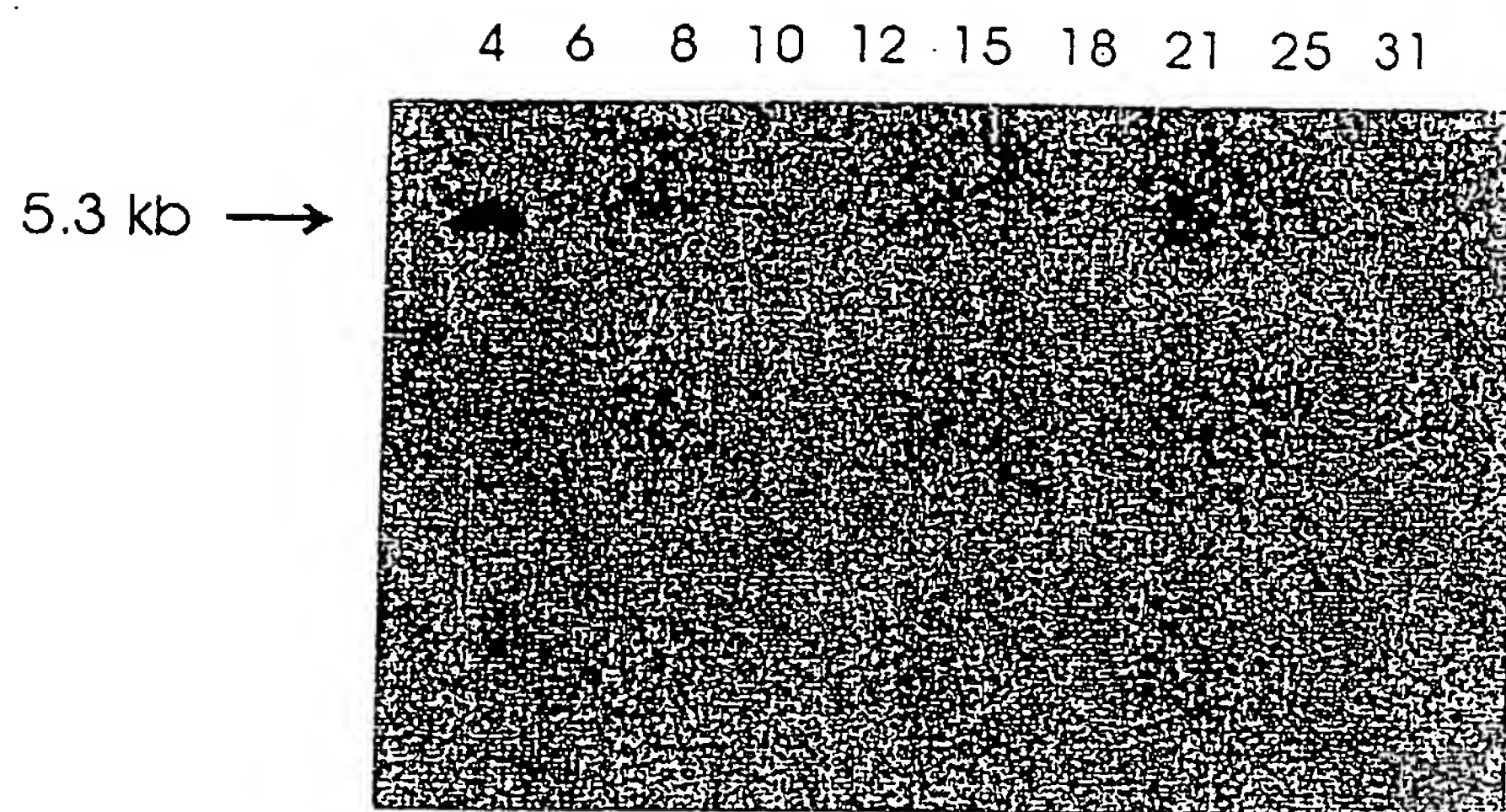
	1401					1450
wSSIII	GAHYIGKAMT	YCDKATTVSP	TYSRDVAGHG	AIAPHREKFY	GILNGIDPDI	
mSSIII	GAHHIGKAMR	YCDKATTVSN	TYSKEVSGHG	AIVPHLGKFY	GILNGIDPDI	
pSSIII	GADLIGRAMT	NADKATTVSP	TYSQEVSGNP	VIAPHLHKFH	GIVNGIDPDI	
	1451					1500
wSSIII	WDPYTDNFIP	VPYTCENVVE	GKRAAKRALQ	QKFGQQTDV	PIVGIIITRLT	
mSSIII	WDPYNDNFIP	VHYTCENVVE	GKRAAKRALQ	QKFGQQIDV	PVVGIVTRLT	
pSSIII	WDPLNDKFIP	IPYTSENVVE	GKTAAKEALQ	RKLGLKQADL	PLVGIIITRLT	
	1501					1550
wSSIII	AQKGIHLIKH	AIHRTLESNG	HVLLGSAPD	HRIQGDFCRL	ADALHGVYHG	
mSSIII	AQKGIHLIKH	AIHRTLERNG	QVLLGSAPD	SRIQADFVNL	ANTLHGVNHG	
pSSIII	HQKGIHLIKH	AIWRTLERNG	QVLLGSAPD	PRVQNNFVNL	ANQLHSKYND	
	1551					1600
wSSIII	RVKLVLTUDE	PLSHLIYAGS	DFIIVPSIFE	PCGLTQLVAM	RYGSIPIVRK	
mSSIII	QVRLSLTYDE	PLSHLIYAGS	DFILVPSIFE	PCGLTQLVAM	RYGTIPIVRK	
pSSIII	RARLCLTYDE	PLSHLIYAGA	DFILVPSIFE	PCGLTQLTAM	RYGSIPVVRK	

FIGURE 7H

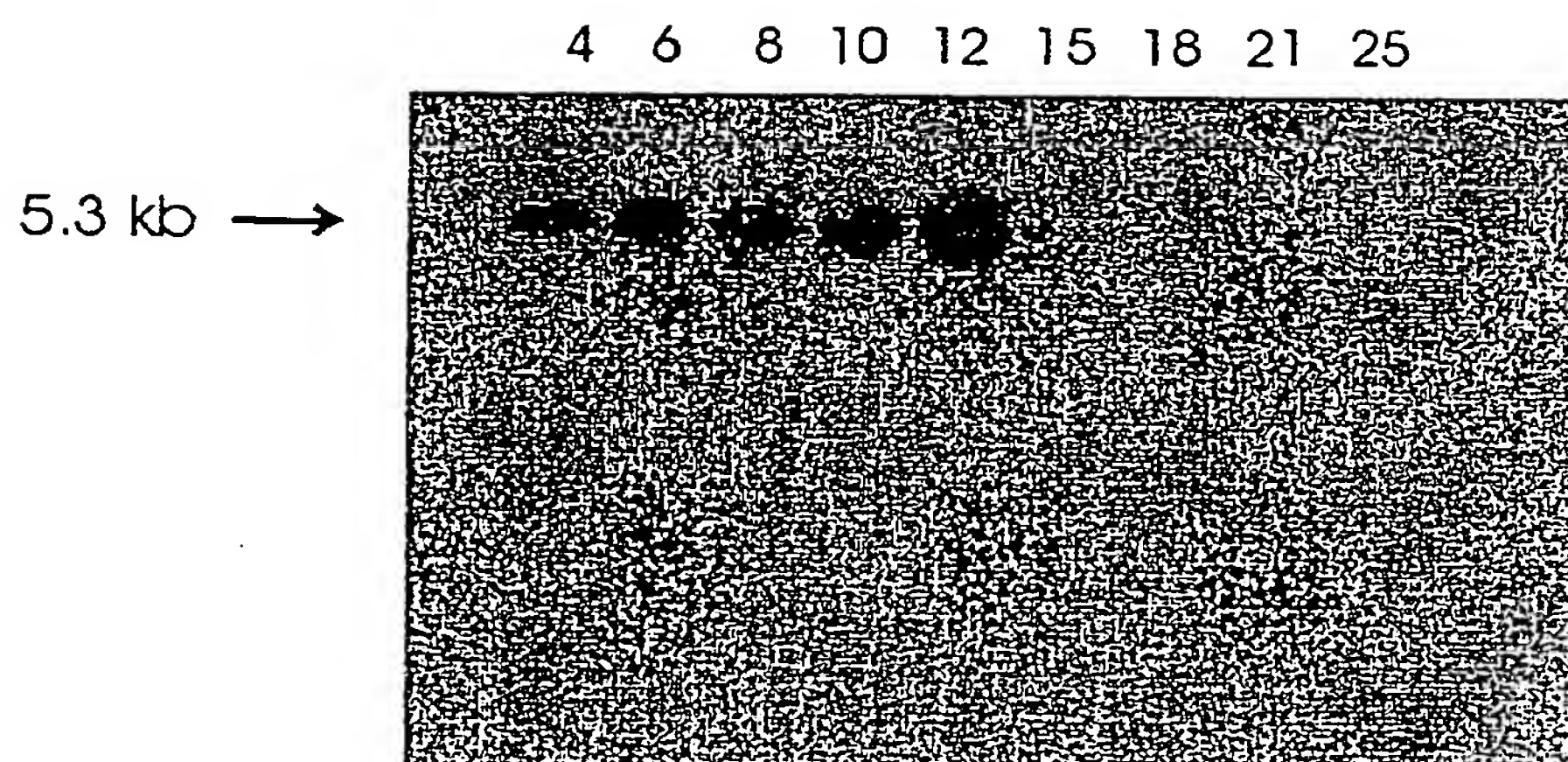
1601					1650
wSSIII	TGGLHDTVFD	VDNDKDRARS	LGLEPNGFSF	DGADSNGV DY	ALNRAIGAWF
mSSIII	TGGLFDTVFD	VDNDKERARD	RGLEPNGFSF	DGADSNGV DY	ALNRAISAWF
pSSIII	TGGLYDTVFD	VDHDKERAQQ	CGLEPNGFSF	DGADAGGV DY	ALNRALSAWY
1651				1689	
wSSIII	DARDWFHSLC	KRVMEQDWSW	NRPALDYIEL	YHAARKF*~	
mSSIII	DARSWFHSLC	KRVMEQDWSW	NRPALDYIEL	YRSASKL~~	
pSSIII	DGRDWFNSLC	KQVMEQDWSW	NRPALDYLEL	YHAARKLE*	

FIGURE 7I

**[a] Wyuna**



**[b] Gabo**



**[c] Gabo**



**FIGURE 8**

FIGURE 9A	FIGURE 9B
FIGURE 9C	FIGURE 9D
FIGURE 9E	FIGURE 9F

FIGURE 9

	Region 1			Region 2	
	10	20	30	40	50
wGBSS	81 FVGAEMAPWS	KTGGLGDLG	GLPPAMAANG	HRVMVISPRY	DQYKDAWDT-
wSS1	144 -*TG*A**YA	*S*****VC*	S**I*L**R*	* ** ** VM**	LNGSSDKNYA
wSS2	314 --A**CS**C	* ** ** VA*	A**K*L*KR*	* ** ** VV**	GD*EE*Y*V-
wSS3	1187 -IAV***VA	*V*****VVT	S*SR*IQDL*	*T*E**L*K*	*CLNQSSVK-

wGBSS	171 LEKVRGKTKE	KIYGPDA GTD	YEDNQQRFSL	LCQAALVPR	ILNLDNNPYF
wSS1	234 -HRPGSLYGD	-----NFGA	FG**F*YT*	**Y**C*A*L	**E*GGYI*G
wSS2	404 RHRQEDIYGG	-----S	RQEIMK*MI*	F*K**V**W	HVPCGGV**G
wSS3	1277 **PQN*MFGV	-----GCVY	GRNDDR**GF	F*HS***--F	**QNEFS*H-

wGBSS	261 FCIHNISYQG	RFSFDDEAQL	NLPD-----R	EKSSFDFIDG	YDKPVEGRKI
wSS1	324 LV***LAH**	LEPASTYPD*	G**PEWYGAL	EWVFPWEARR	HALDKGEAVN
wSS2	494 MV***AH**	*GPV*E*PFT	E**-----	-EHYLEHFR	**PVGGEHAN
wSS3	1367 *T***L-EF*	AHYIGKAMTY	CDK-----	-----	-----

FIGURE 9A

60	70	80	90	
-----SVVSE	IKVVDKYERV	RYFHCYKRGV	DRVFDHPCF	170
KALYTGKHIK	*PCFGGSHE*	TF**E*RDN*	*W*****SY	233
-----G*RKY	Y*AAGQDME*	N***A*ID**	*F**I*A*L*	403
-----	-DLHLYQSFS	WGGTEI*VW*	G**EDLTVY*	1276

Region 3

150	160	170	180	
SGPYGEDVVF	VCNDWHTGLL	ACYLKSNIQS	NGIYRAAKVA	260
QN-----CM*	*V***AS*V	PVL*AAK*RP	Y*V**DSRST	323
D*-----NL**	IA*****A**	PV***AY*RD	H*LMQYTRSI	493
-----II	H*H**SSAPV	*WLY*EH*SQ	-SRMASTR*V	1366

240	250	260	270	
NWMKAGILQA	DKVLTVSPYY	AEELISGEAR	GCELDNIMRL	350
FLKG*VVTAD	RI*TVSQG*S	W*VTTAEGGQ	*LNELLSS*K	413
YFAAGLKMAD	QV*VVSPG*L	W*LKTVEGGW	*LHDIIRQND	583
-----	-----AT	TVSPTYSRDV	AGHGAIAPHR	1456

FIGURE 9B



Region 4				
	280	290	300	310 320
wGBSS	351	TGITTVNGM DVSEWDPTKD	KFLAVNYDIT	TALEGKALNK EALEGKALNK
wSS1	414	<u>SVLNG***I *IND*N**T*</u>	<u>*C*PHH*SV-</u>	<u>-----DD*S***KC*</u>
wSS2	584	<u>WKTRG***I *NM**N*EV*</u>	<u>VH*KSDGYTN</u>	<u>-----FSLG TLDS**RQC*</u>
wSS3	1457	<u>EKFYG*L*I *PDI***YT*</u>	<u>N*IP*P*TCE</u>	<u>-----NVVEG* **AKRALQQ*</u>

Region 5a				
	370	380	390	400 410
wGBSS	441	LKEEDVQIVL LGTGKKKFER	LLKSIEEKFP	SKVRAVVRFN -----APLA
wSS1	504	<u>*MR***F*M **S*DPI**G</u>	<u>WMR*T*SSYK</u>	<u>D*F*GW*G*S -----V*VS</u>
wSS2	674	<u>V-SQ***L*M ***RHDLS</u>	<u>M*RHF*REHH</u>	<u>D***GW*G*S -----VR**</u>
wSS3	1547	<u>TL*SNG*V** **SAPDHRIQ</u>	<u>GDFCRLADAL</u>	<u>HG*YHGRVKL -VLTUDE**S</u>

FIGURE 9C

Region 5			
330	340	350	360
EALQAEVGLP	VDRKVPLVAF	IGRLEEQKGP	DVMIASIP EI 440
AE**K*L**	*RED**IG*	***DY***I	*LIKMA***- 503
***R*L**Q	*RAD**LG*	***DG***V	EIIADAM*W* 673
FG**QT----	---D**I*GI	*T***TA***I	-HL*KHAIHR 1546

Region 6			
420	430	440	450
HQMMAGADVL	AVTSRFEPCG	LIQLQGMRYG	TPCACASTGG 530
*RIT**C*I*	LMP*****	*N**YA*Q**	*VPVVHG*** 593
*RIT***A*	LMP*****	*N**YA*A**	*VPVVHAV** 763
*LIY**S*FI	I*P*I*****	*T***VA***	SIPIVRK*** 1636

Region 7			
530	593	763	1636

FIGURE 9D

45/50

Region 7 (Continued)

460 470 480 490 500

WGBSS 531 LVDTIVEGKT GFHMGRLSYD CNVVEPADVK KVVTTLKRAV KVVGTPAYIIE  
WSS1 594 \*R\*-\*\*TFN ----- --PFGAKGEE GTGWAFSPLT VDKMLW\*LRT  
WSS2 764 VR\*-\*\*PPFD ----- --PENHSGLG ---W\*FD\*\*E AHKLIE\*LGH  
WSS3 1637 \*\*\*-\*FDVNDKDRAR\*LG LEPNGFSFDG ADSNGVDY\*L NRAIGAWFDA

550 560 570 580 590 600  
WGBSS 621 APLAMENVAA P\* .....  
WSS1 684 FVDQPYVM.. .....  
WSS2 854 KYQW.....  
WSS3 1727 .....

FIGURE 9E

510	520	530	540	
MVKNCMIQDL	SWKGPAKNWE	DVLELGVEG	SEPGIVGEEI	620
AMSTFREHKP	**E*LM*RGM	TKDHTWDHAA	EQYEQIF*WA	683
CLRTYRDYKE	**R*LQERGM	SQDFSWEHAA	KLYED*LLKA	853
RDWFHSLCKK	VMEQDWSWNR	PA*DYIELYH	AARKF*....	1726
610	620	630		
.....	.....	.....	.....	710
.....	.....	.....	.....	773
.....	.....	.....	.....	943
.....	.....	.....	.....	1816

FIGURE 9F

47/50

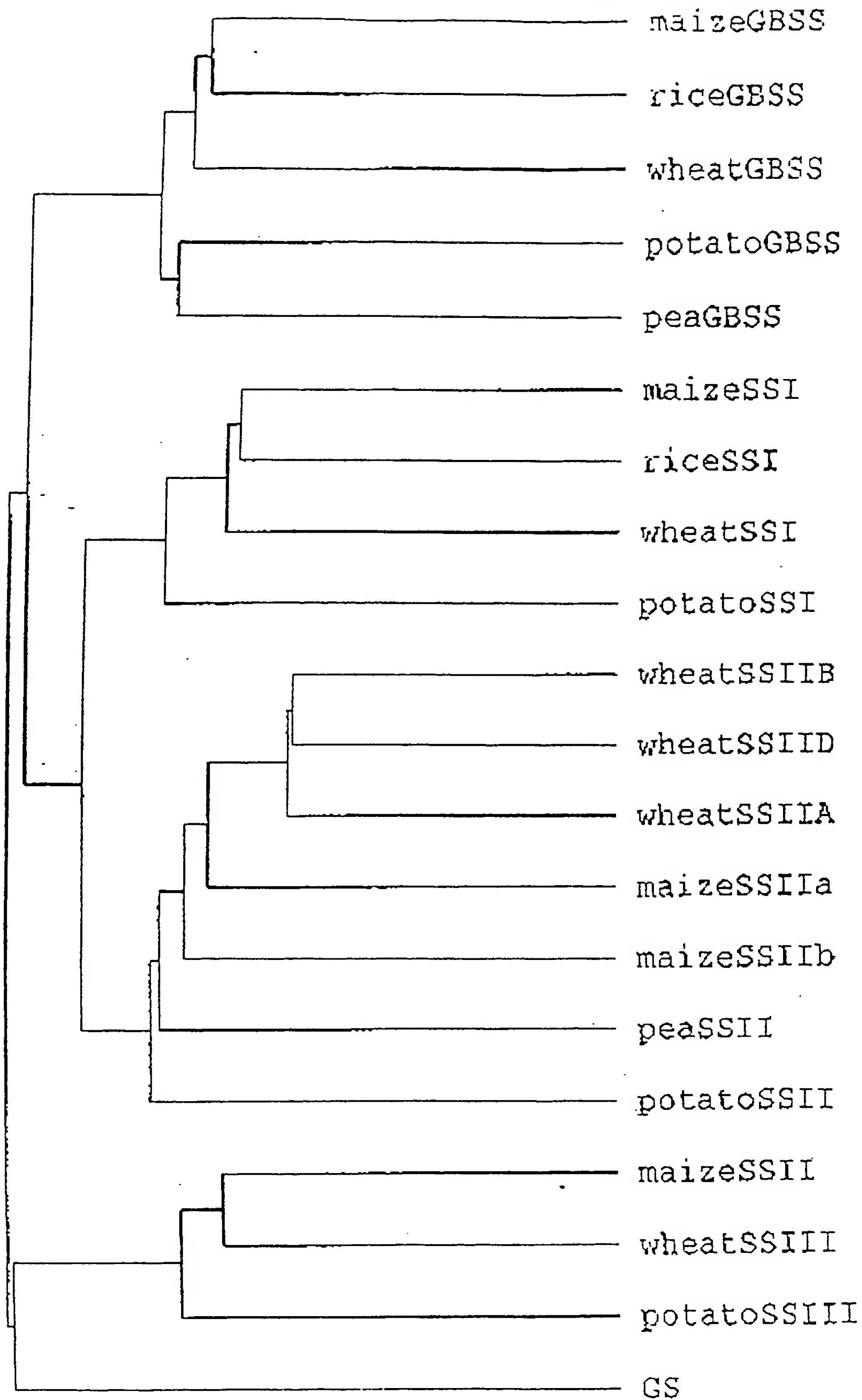


FIGURE 10

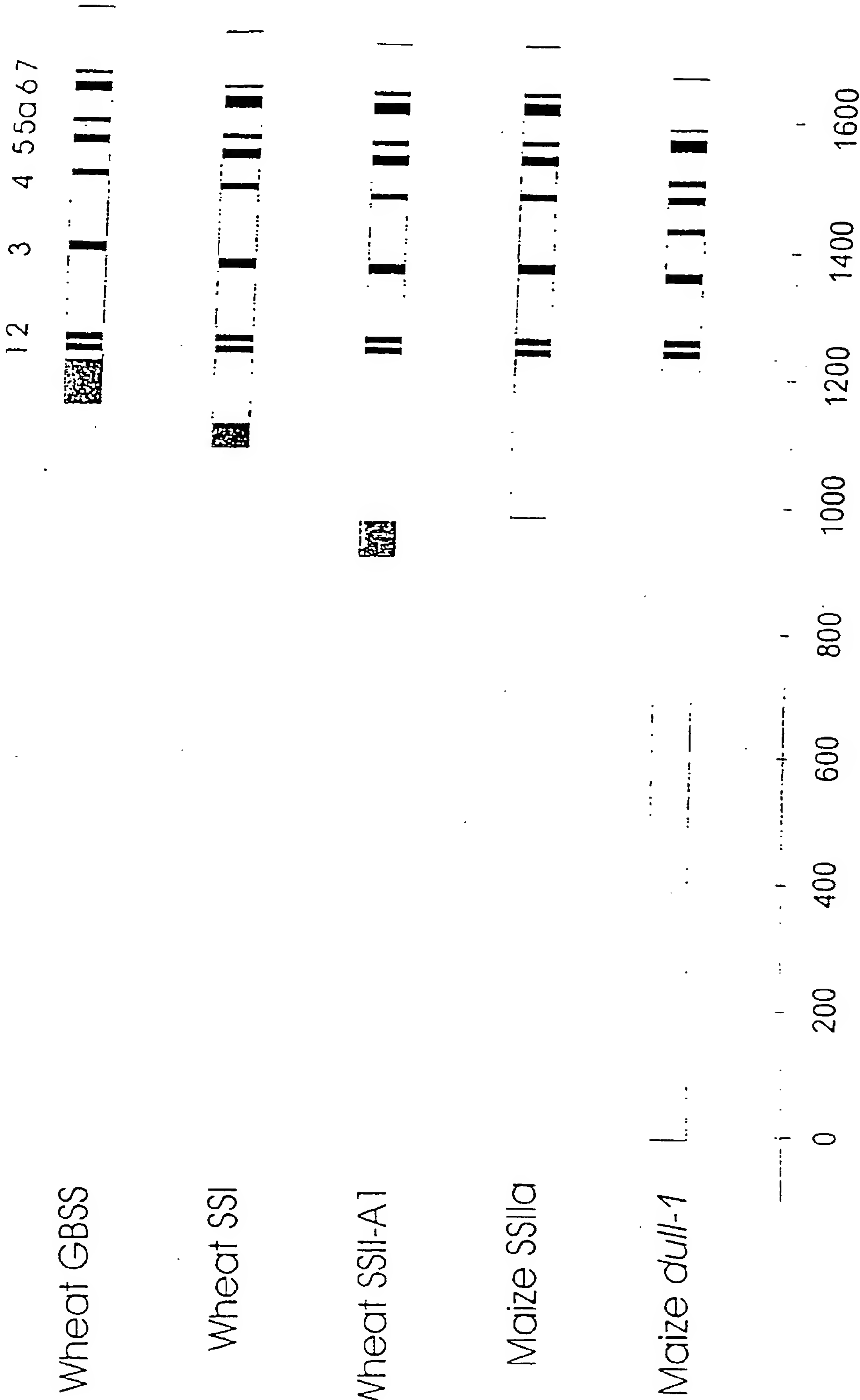


FIGURE 11



SSIII cDNA

cDNA sequences not in  
the genomic sequence

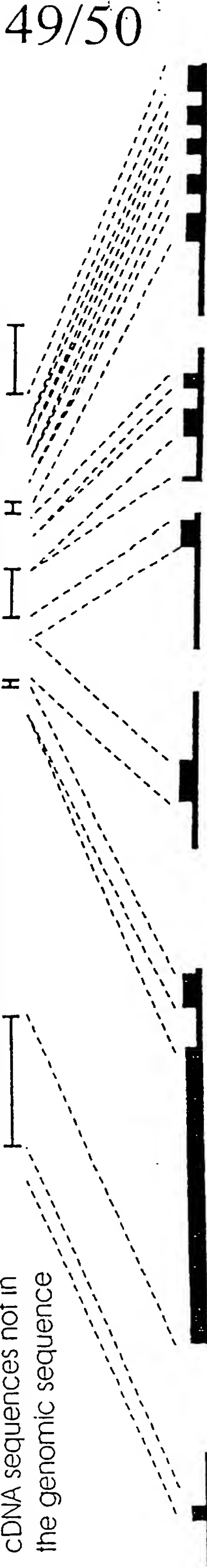


FIGURE 12

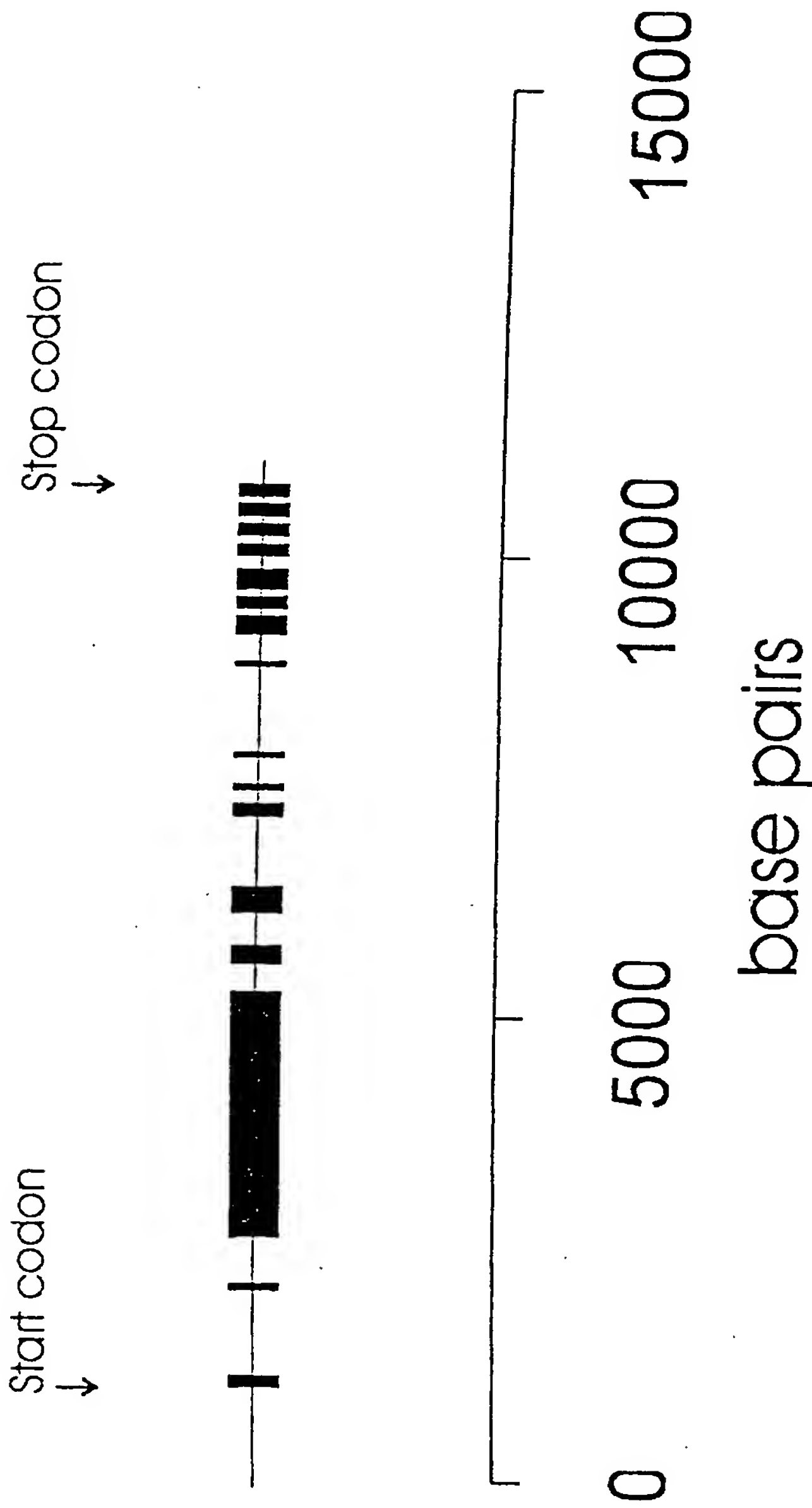


FIGURE 13

- 1 -

## SEQUENCE LISTING

<110> COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION  
GOODMAN FIELDER LIMITED  
GROUPE LIMAGRAIN PACIFIC PTY LTD

<120> NOVEL GENES ENCODING WHEAT STARCH SYNTHASES AND USES  
THEREFOR

<130> p:\oper\mro\pi-wss.pct

<140> TO BE ADVISED

<141> 2000-04-28

<150> AU PQ0052/99

<151> 1999-04-29

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cccactgccg cgctactccc cactcccact gccaccacct ccgcctgcgc cgcgctcttg 120  
gcggaccaac ccgcgcacgc tatcacgac acccaccgcc atcccggccg ccgcc atg 178  
Met  
1

tcg tcg gcg gtc gcg tcc gcc gcg tcc ttc ctc gcg ctc gcg tcc gcc 226  
Ser Ser Ala Val Ala Ser Ala Ala Ser Phe Leu Ala Leu Ala Ser Ala  
5 10 15

tcc ccc ggg aga tca cgg agg agg acg agg gtg agc gcg tcg cca ccc 274  
Ser Pro Gly Arg Ser Arg Arg Arg Thr Arg Val Ser Ala Ser Pro Pro  
20 25 30

cac acc ggg gct ggc agg ttg cac tgg ccg ccg tcg ccg ccg cag cgc 322  
His Thr Gly Ala Gly Arg Leu His Trp Pro Pro Ser Pro Pro Gln Arg  
35 40 45

acg gct cgc gac gga gcg gtg gcc gcg cgc gcc gcc ggg aag aag gac 370  
Thr Ala Arg Asp Gly Ala Val Ala Ala Arg Ala Ala Gly Lys Lys Asp  
50 55 60 65

gcg ggg atc gac gac gcc gcg ccc gcg agg cag ccc cgc gca ctc cgc 418  
Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu Arg  
70 75 80

ggt ggc gcc gcc acc aag gtt gcg gag cgg agg gat ccc gtc aag acg 466  
Gly Gly Ala Ala Thr Lys Val Ala Glu Arg Arg Asp Pro Val Lys Thr  
85 90 95

ctc gat cgc gac gcc gcg gaa ggt ggc gcg ccg tcc ccg ccg gca ccg 514  
Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ser Pro Pro Ala Pro

- 2 -

100	105	110	
agg cag gag gac gcc cgt ctg ccg agc atg aac ggc atg ccg gtg aac			562
Arg Gln Glu Asp Ala Arg Leu Pro Ser Met Asn Gly Met Pro Val Asn			
115	120	125	
ggt gaa aac aaa tct acc ggc ggc ggc ggc gcg act aaa gac agc ggg			610
Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp Ser Gly			
130	135	140 145	
ctg ccc gca ccc gca cgc gcg ccc cag ccg tcg agc cag aac aga gta			658
Leu Pro Ala Pro Ala Arg Ala Pro Gln Pro Ser Ser Gln Asn Arg Val			
	150	155 160	
ccg gtg aat ggt gaa aac aaa gct aac gtc gcc tcg ccg ccg acg agc			706
Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr Ser			
	165	170 175	
ata gcc gag gtc gcg gct ccg gat ccc gca gct acc att tcc atc agt			754
Ile Ala Glu Val Ala Ala Pro Asp Pro Ala Ala Thr Ile Ser Ile Ser			
	180	185 190	
gac aag gcg cca gag tcc gtt gtc cca gcc gag aag gcg ccg ccg tcg			802
Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Ala Pro Pro Ser			
	195	200 205	
tcc ggc tca aat ttc gtg ccc tcg gct tct gct ccc ggg tct gac act			850
Ser Gly Ser Asn Phe Val Pro Ser Ala Ser Ala Pro Gly Ser Asp Thr			
210	215	220 225	
gtc agc gac gtg gaa ctt gaa ctg aag aag ggt gcg gtc att gtc aaa			898
Val Ser Asp Val Glu Leu Glu Leu Lys Lys Gly Ala Val Ile Val Lys			
	230	235 240	
gaa gct cca aac cca aag gct ctt tcg ccg ccc gca gca ccc gct gta			946
Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala Val			
	245	250 255	
caa caa gac ctt tgg gac ttc aag aaa tac att ggt ttc gag gag ccc			994
Gln Gln Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu Pro			
	260	265 270	
gtg gag gcc aag gat gat ggc cgg gct gtt gca gat gat gcg ggc tcc			1042
Val Glu Ala Lys Asp Asp Gly Arg Ala Val Ala Asp Asp Ala Gly Ser			
	275	280 285	
ttc gaa cac cac cag aat cac gat tcc ggg cct ttg gca ggg gag aac			1090
Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly Glu Asn			
290	295	300 305	
gtc atg aac gtg gtc gtc gtg gct gct gaa tgt tct ccc tgg tgc aaa			1138
Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp Cys Lys			
	310	315 320	
aca ggt ggt ctt gga gat gtt gcc ggt gct ttg ccc aag gct ttg gcg			1186
Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala Leu Ala			
	325	330 335	
aag aga gga cat cgt gtt atg gtt gtg gta cca agg tat ggg gac tat			1234
Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly Asp Tyr			
	340	345 350	
gag gaa gcc tac gat gtc gga gtc cga aaa tac tac aag gct gct gga			1282
Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala Ala Gly			
	355	360 365	

- 3 -

cag gat atg gaa gtg aat tat ttc cat gct tat atc gat gga gtt gat Gln Asp Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly Val Asp 370 375 380 385	1330
ttt gtg ttc att gac gct cct ctc ttc cga cac cgc cag gaa gac att Phe Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Glu Asp Ile 390 395 400	1378
tat ggg ggc agc aga cag gaa att atg aag cgc atg att ttg ttc tgc Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe Cys 405 410 415	1426
aag gcc gct gtc gag gtt cca tgg cac gtt cca tgc ggc ggt gtc cct Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val Pro 420 425 430	1474
tat ggg gat gga aat ctg gtg ttt att gca aat gat tgg cac acg gca Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr Ala 435 440 445	1522
ctc ctg cct gtc tat ctg aaa gca tat tac agg gac cat ggt ttg atg Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu Met 450 455 460 465	1570
cag tac act cgg tcc att atg gtg ata cat aac atc gct cac cag ggc Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His Gln Gly 470 475 480	1618
cgt ggc cca gta gat gag ttc ccg ttc acc gag ttg cct gag cac tac Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu His Tyr 485 490 495	1666
ctg gaa cac ttc aga ctg tac gac ccc gtg ggt ggt gaa cac gcc aac Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His Ala Asn 500 505 510	1714
tac ttc gcc gcc ggc ctg aag atg gcg gac cag gtt gtc gtc gtg agc Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val Ser 515 520 525	1762
ccg ggg tac ctg tgg gag ctg aag acg gtg gag ggc ggc tgg ggg ctt Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp Gly Leu 530 535 540 545	1810
cac gac atc ata cgg cag aac gac tgg aag acc cgc ggc atc gtg aac His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile Val Asn 550 555 560	1858
ggc atc gac aac atg gag tgg aac ccc gag gtg gac gtc cac ctc aag Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Val His Leu Lys 565 570 575	1906
tcg gac ggc tac acc aac ttc tcc ctg ggg acg ctg gac tcc ggc aag Ser Asp Gly Tyr Thr Asn Phe Ser Leu Gly Thr Leu Asp Ser Gly Lys 580 585 590	1954
cgg cag tgc aag gag gcc ctg cag cgg gag ctg ggc ctg cag gtc cgc Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln Val Arg 595 600 605	2002
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- 4 -

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gtg cag ctg gtc atg ctg ggc acc ggg cgc cac gac ctg gag ggc atg 2146  
 Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu Gly Met  
 645 650 655

ctg cgg cac ttc gag cgg gag cac cac gac aag gtg cgc ggg tgg gtg 2194  
 Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp Val  
 660 665 670

ggg ttc tcc gtg cgg ctg gcg cac cgg atc acg gcc ggc gcc gac gcg 2242  
 Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp Ala  
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ctc ctc atg ccc tcc cgg ttc gag ccg tgc gga ctg aac cag ctc tac 2290  
 Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr  
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gcc atg gcc tac ggc acc gtc ccc gtc gtg cat gcc gtc ggt ggc ctg 2338  
 Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly Leu  
 710 715 720

agg gac acc gtg ccg ccg ttc gac ccc ttc aac cac tcc ggg ctc ggg 2386  
 Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly Leu Gly  
 725 730 735

tgg acg ttc gac cgc gca gag gcg cag aag ctg atc gag gcg ctc ggg 2434  
 Trp Thr Phe Asp Arg Ala Glu Ala Gln Lys Leu Ile Glu Ala Leu Gly  
 740 745 750

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 His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg Gly Leu  
 755 760 765

cag gag cgc ggc atg tcg cag gac ttc agc tgg gag cat gcc gcc aag 2530  
 Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala Ala Lys  
 770 775 780 785

ctc tac gag gac gtc ctc gtc aag gcc aag tac cag tgg tgaacgctag 2579  
 Leu Tyr Glu Asp Val Leu Val Lys Ala Lys Tyr Gln Trp  
 790 795

ctgctagccg gtccagcccc gcatgcgtgc atgacaggat ggaattgcgc attgcgcacg 2639  
 caggaagggtg ccatggagcg ccggcatccg cgaagtacag tgacatgagg tgtgtgtggt 2699  
 tgagacgctg attccgatct ggtccgtagc agagtagagc ggaggtaggg aagcgctcct 2759  
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 Arg Thr Ala Arg Asp Gly Ala Val Ala Ala Arg Ala Ala Gly Lys Lys  
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 Asp Ala Gly Ile Asp Asp Ala Ala Pro Ala Arg Gln Pro Arg Ala Leu  
 65 70 75 80  
 Arg Gly Gly Ala Ala Thr Lys Val Ala Glu Arg Arg Asp Pro Val Lys  
 85 90 95  
 Thr Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ser Pro Pro Ala  
 100 105 110  
 Pro Arg Gln Glu Asp Ala Arg Leu Pro Ser Met Asn Gly Met Pro Val  
 115 120 125  
 Asn Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp Ser  
 130 135 140  
 Gly Leu Pro Ala Pro Ala Arg Ala Pro Gln Pro Ser Ser Gln Asn Arg  
 145 150 155 160  
 Val Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr  
 165 170 175  
 Ser Ile Ala Glu Val Ala Ala Pro Asp Pro Ala Ala Thr Ile Ser Ile  
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 Ser Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Ala Pro Pro  
 195 200 205  
 Ser Ser Gly Ser Asn Phe Val Pro Ser Ala Ser Ala Pro Gly Ser Asp  
 210 215 220  
 Thr Val Ser Asp Val Glu Leu Glu Leu Lys Lys Gly Ala Val Ile Val  
 225 230 235 240  
 Lys Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala  
 245 250 255  
 Val Gln Gln Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu  
 260 265 270  
 Pro Val Glu Ala Lys Asp Asp Gly Arg Ala Val Ala Asp Asp Ala Gly  
 275 280 285  
 Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly Glu  
 290 295 300  
 Asn Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp Cys  
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 Lys Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala Leu  
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 Ala Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly Asp  
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 Tyr Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala Ala

- 6 -

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Gly Gln Asp Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly Val				
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Asp Phe Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Glu Asp				
385		390		400
Ile Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu Phe				
	405		410	415
Cys Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly Val				
	420		425	430
Pro Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His Thr				
	435		440	445
Ala Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly Leu				
	450		455	460
Met Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His Gln				
	465		470	475
Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu His				
	485		490	495
Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His Ala				
	500		505	510
Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val Val				
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Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp Gly				
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Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile Val				
	545		550	555
Asn Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Val His Leu				
	565		570	575
Lys Ser Asp Gly Tyr Thr Asn Phe Ser Leu Gly Thr Leu Asp Ser Gly				
	580		585	590
Lys Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln Val				
	595		600	605
Arg Gly Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly Gln				
	610		615	620
Lys Gly Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser Gln				
	625		630	635
Asp Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu Gly				
	645		650	655
Met Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly Trp				
	660		665	670
Val Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala Asp				
	675		680	685
Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu				
	690		695	700

- 7 -

Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly  
705 710 715 720

Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly Leu  
725 730 735

Gly Trp Thr Phe Asp Arg Ala Glu Ala Gln Lys Leu Ile Glu Ala Leu  
740 745 750

Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg Gly  
755 760 765

Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala Ala  
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785 790 795

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Met Ser Ser Ala Val Ala Ser Ala  
1 5

gcg tcc ttc ctc gcg ctc gcc tcc gcc tcc ccc ggg aga tca cgc agg 160  
Ala Ser Phe Leu Ala Leu Ala Ser Ala Ser Pro Gly Arg Ser Arg Arg  
10 15 20

cgg gcg agg gtg agc gcg ccg cca ccc cac gcc ggg gcc ggc agg ctg 208  
Arg Ala Arg Val Ser Ala Pro Pro Pro His Ala Gly Ala Gly Arg Leu  
25 30 35 40

cac tgg ccg ccg tgg ccg ccg cag cgc acg gct cgc gac gga ggt gtg 256  
His Trp Pro Pro Trp Pro Pro Gln Arg Thr Ala Arg Asp Gly Gly Val  
45 50 55

gcc gcg cgc gcc gcc ggg aag aag gac gcg agg gtc gac gac gac gcc 304  
Ala Ala Arg Ala Ala Gly Lys Lys Asp Ala Arg Val Asp Asp Ala Ala  
60 65 70

gcg tcc gcg agg cag ccc cgc gca cgc cgc ggt ggc gcc gcc acc aag 352  
Ala Ser Ala Arg Gln Pro Arg Ala Arg Arg Gly Gly Ala Ala Thr Lys  
75 80 85

gtc gcg gag cgg agg gat ccc gtc aag acg ctc gat cgc gac gcc gcg 400  
Val Ala Glu Arg Arg Asp Pro Val Lys Thr Leu Asp Arg Asp Ala Ala  
90 95 100

gaa ggt ggc gcg ccg gca ccg ccg gca ccg agg cag gac gcc gcc cgt 448  
Glu Gly Gly Ala Pro Ala Pro Pro Ala Pro Arg Gln Asp Ala Ala Arg  
105 110 115 120

cca ccg agt atg aac ggc acg ccg gtg aac ggt gag aac aaa tct acc 496  
Pro Pro Ser Met Asn Gly Thr Pro Val Asn Gly Glu Asn Lys Ser Thr

- 8 -

125	130	135	
ggc ggc ggc ggc ggc acc aaa gac agc ggg ctg ccc gca ccc gca cgc Gly Gly Gly Gly Ala Thr Lys Asp Ser Gly Leu Pro Ala Pro Ala Arg 140 145 150			544
gcg ccc cat ccg tcg acc cag aac aga gta cca gtg aac ggt gaa aac Ala Pro His Pro Ser Thr Gln Asn Arg Val Pro Val Asn Gly Glu Asn 155 160 165			592
aaa gct aac gtc gcc tcg ccg ccg acg agc ata gcc gag gtc gtg gct Lys Ala Asn Val Ala Ser Pro Pro Thr Ser Ile Ala Glu Val Val Ala 170 175 180			640
ccg gat tcc gca gct acc att tcc atc agt gac aag gcg ccg gag tcc Pro Asp Ser Ala Ala Thr Ile Ser Ile Ser Asp Lys Ala Pro Glu Ser 185 190 195 200			688
gtt gtc cca gcc gag aag ccg ccg ccg tcg tcc ggc tca aat ttc gtg Val Val Pro Ala Glu Lys Pro Pro Pro Ser Ser Gly Ser Asn Phe Val 205 210 215			736
gtc tcg gct tct gct ccc agg ctg gac att gac agc gat gtt gaa cct Val Ser Ala Ser Ala Pro Arg Leu Asp Ile Asp Ser Asp Val Glu Pro 220 225 230			784
gaa ctg aag aag ggt gcg gtc atc gtc gaa gaa gct cca aac cca aag Glu Leu Lys Lys Gly Ala Val Ile Val Glu Glu Ala Pro Asn Pro Lys 235 240 245			832
gct ctt tcg ccg cct gca gcc ccc gct gta caa gaa gac ctt tgg gac Ala Leu Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp 250 255 260			880
ttc aag aaa tac att ggc ttc gag gag ccc gtg gag gcc aag gat gat Phe Lys Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp 265 270 275 280			928
ggc tgg gct gtt gca gat gat gcg ggc tcc ttt gaa cat cac cag aac Gly Trp Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn 285 290 295			976
cat gat tcc gga cct ttg gca ggg gag aac gtc atg aac gtg gtc gtc His Asp Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val 300 305 310			1024
gtg gct gct gaa tgt tct ccc tgg tgc aaa aca ggt ggt ctt gga gat Val Ala Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp 315 320 325			1072
gtt gcc ggt gct ttg ccc aag gct ttg gcg aag aga gga cat cgt gtt Val Ala Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val 330 335 340			1120
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gga gtc cga aaa tac tac aag gct gct gga cag gat atg gaa gtg aat Gly Val Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn 365 370 375			1216
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- 9 -

cct ctc ttc cga cac cgc cag gaa gac att tat ggg ggc agc aga cag Pro Leu Phe Arg His Arg Gln Glu Asp Ile Tyr Gly Gly Ser Arg Gln 395 400 405	1312
gaa att atg aag cgc atg att ttg ttc tgc aag gcc gct gtc gag gtt Glu Ile Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val 410 415 420	1360
cct tgg cac gtt cca tgc ggc ggt gtc cct tat ggg gat gga aat ctg Pro Trp His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu 425 430 435 440	1408
gtg ttt att gca aat gat tgg cac acg gca ctc ctg cct gtc tat ctg Val Phe Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu 445 450 455	1456
aaa gca tat tac agg gac cat ggt ttg atg cag tac act cgg tcc att Lys Ala Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile 460 465 470	1504
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ttc ccg ttc acc gag ttg cct gag cac tac ctg gaa cac ttc aga ctg Phe Pro Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu 490 495 500	1600
tac gac ccc gtg ggt ggt gag cac gcc aac tac ttc gcc gcc ggc ctg Tyr Asp Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu 505 510 515 520	1648
aag atg gcg gac cag gtt gtc gtg gtg agc ccc ggg tac ctg tgg gag Lys Met Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu 525 530 535	1696
ctc aag acg gtg gag ggc ggc tgg ggg ctt cac gac atc ata cgg cag Leu Lys Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln 540 545 550	1744
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ggc ttc atc ggc cgc ctg gac ggg cag aag ggc gtg gag atc atc gcg Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile Ala 620 625 630	1984
gac gcc atg ccc tgg atc gtg agc cag gac gtg cag ctg gtc atg ctg Asp Ala Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu 635 640 645	2032

- 10 -

ggc acc ggc cgc cac gac ctg gag agc atg ctg cgg cac ttc gag cgg 2080  
 Gly Thr Gly Arg His Asp Leu Glu Ser Met Leu Arg His Phe Glu Arg  
 650 655 660

gag cac cac gac aag gtg cgc ggg tgg gtg ggg ttc tcc gtg cgc ctg 2128  
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 665 670 675 680

gcg cac cgg atc acg gcg ggc gcc gac gcg ctc ctc atg ccc tcc cgg 2176  
 Ala His Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg  
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 Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr  
 700 705 710

gtc ccc gtc gtg cac gcc gtc ggc ggg gtg agg gac acc gtg ccg ccg 2272  
 Val Pro Val Val His Ala Val Gly Gly Val Arg Asp Thr Val Pro Pro  
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gag gcg cac aag ctg atc gag gcg ctc ggg cac tgc ctc cgc acc tac 2368  
 Glu Ala His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr  
 745 750 755 760

cgg gac tac aag gag agc tgg agg ggc ctc cag gag cgc ggc atg tcg 2416  
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 50 55 60  
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 85 90 95  
 Lys Thr Leu Asp Arg Asp Ala Ala Glu Gly Gly Ala Pro Ala Pro Pro  
 100 105 110  
 Ala Pro Arg Gln Asp Ala Ala Arg Pro Pro Ser Met Asn Gly Thr Pro  
 115 120 125  
 Val Asn Gly Glu Asn Lys Ser Thr Gly Gly Gly Gly Ala Thr Lys Asp  
 130 135 140  
 Ser Gly Leu Pro Ala Pro Ala Arg Ala Pro His Pro Ser Thr Gln Asn  
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 Arg Val Pro Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro  
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 180 185 190  
 Ile Ser Asp Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Pro Pro  
 195 200 205  
 Pro Ser Ser Gly Ser Asn Phe Val Val Ser Ala Ser Ala Pro Arg Leu  
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 Asp Ile Asp Ser Asp Val Glu Pro Glu Leu Lys Lys Gly Ala Val Ile  
 225 230 235 240  
 Val Glu Glu Ala Pro Asn Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro  
 245 250 255  
 Ala Val Gln Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu  
 260 265 270  
 Glu Pro Val Glu Ala Lys Asp Asp Gly Trp Ala Val Ala Asp Asp Ala  
 275 280 285  
 Gly Ser Phe Glu His His Gln Asn His Asp Ser Gly Pro Leu Ala Gly  
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 Glu Asn Val Met Asn Val Val Val Val Ala Ala Glu Cys Ser Pro Trp  
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 Cys Lys Thr Gly Gly Leu Gly Asp Val Ala Gly Ala Leu Pro Lys Ala  
 325 330 335  
 Leu Ala Lys Arg Gly His Arg Val Met Val Val Val Pro Arg Tyr Gly  
 340 345 350  
 Asp Tyr Glu Glu Ala Tyr Asp Val Gly Val Arg Lys Tyr Tyr Lys Ala  
 355 360 365  
 Ala Gly Gln Asp Met Glu Val Asn Tyr Phe His Ala Tyr Ile Asp Gly  
 370 375 380



- 12 -

Val Asp Phe Val Phe Ile Asp Ala Pro Leu Phe Arg His Arg Gln Glu  
 385 390 395 400  
 Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile Met Lys Arg Met Ile Leu  
 405 410 415  
 Phe Cys Lys Ala Ala Val Glu Val Pro Trp His Val Pro Cys Gly Gly  
 420 425 430  
 Val Pro Tyr Gly Asp Gly Asn Leu Val Phe Ile Ala Asn Asp Trp His  
 435 440 445  
 Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala Tyr Tyr Arg Asp His Gly  
 450 455 460  
 Leu Met Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His  
 465 470 475 480  
 Gln Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu  
 485 490 495  
 His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His  
 500 505 510  
 Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val  
 515 520 525  
 Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp  
 530 535 540  
 Gly Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile  
 545 550 555 560  
 Val Asn Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Val His  
 565 570 575  
 Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser Leu Gly Thr Leu Asp Ser  
 580 585 590  
 Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln  
 595 600 605  
 Val Arg Ala Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly  
 610 615 620  
 Gln Lys Gly Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser  
 625 630 635 640  
 Gln Asp Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu  
 645 650 655  
 Ser Met Leu Arg His Phe Glu Arg Glu His His Asp Lys Val Arg Gly  
 660 665 670  
 Trp Val Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala  
 675 680 685  
 Asp Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln  
 690 695 700  
 Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly  
 705 710 715 720  
 Gly Val Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly

- 13 -

725	730	735
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740	745	750
Leu Gly His Cys Leu Arg Thr Tyr Arg Asp Tyr Lys Glu Ser Trp Arg		
755	760	765
Gly Leu Gln Glu Arg Gly Met Ser Gln Asp Phe Ser Trp Glu His Ala		
770	775	780
Ala Lys Leu Tyr Glu Asp Val Leu Leu Lys Ala Lys Tyr Gln Trp		
785	790	795

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gcc tct gct ccc ggg tct gac act gtc agc gac gtg gaa caa gaa ctg	96
Ala Ser Ala Pro Gly Ser Asp Thr Val Ser Asp Val Glu Gln Glu Leu	
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aag aag ggt gcg gtc gtt gtc gaa gaa gct cca aag cca aag gct ctt	144
Lys Lys Gly Ala Val Val Val Glu Glu Ala Pro Lys Pro Lys Ala Leu	
35 40 45	
tcg ccg cct gca gcc ccc gct gta caa gaa gac ctt tgg gat ttc aag	192
Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys	
50 55 60	
aaa tac att ggt ttc gag gag ccc gtg gag gcc aag gat gat ggc cgg	240
Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg	
65 70 75 80	
gct gtc gca gat gat gcg ggc tcc ttt gaa cac cac cag aat cac gac	288
Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp	
85 90 95	
tcc gga cct ttg gca ggg gag aat gtc atg aac gtg gtc gtc gtg gct	336
Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala	
100 105 110	
gct gag tgt tct ccc tgg tgc aaa aca ggt ggt ctg gga gat gtt gcg	384
Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala	
115 120 125	
ggt gct ctg ccc aag gct ttg gca aag aga gga cat cgt gtt atg gtt	432
Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val	
130 135 140	
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Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Pro Thr Asp Val Gly Val	
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Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn Tyr Phe	
165 170 175	
cat gct tat atc gat gga gtt gat ttt gtg ttc att gac gct cct ctc	576
His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp Ala Pro Leu	
180 185 190	
ttc cga cac cga gag gaa gac att tat ggg ggc agc aga cag gaa att	624
Phe Arg His Arg Glu Glu Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile	
195 200 205	
atg aag cgc atg att ttg ttc tgc aag gcc gct gtt gag gtt cca tgg	672
Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val Pro Trp	
210 215 220	
cac gtt cca tgc ggc ggt gtc cct tat ggg gat gga aat ctg gtg ttt	720
His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu Val Phe	
225 230 235 240	
att gca aat gat tgg cac acg gca ctc ctg cct gtc tat ctg aaa gca	768
Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala	
245 250 255	
tat tac agg gac cat ggt ttg atg cag tac act cgg tcc att atg gtg	816
Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile Met Val	
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ata cat aac atc gct cac cag ggc cgt ggc cct gta gat gaa ttc ccg	864
Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro	
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ttc acc gag ttg cct gag cac tac ctg gaa cac ttc aga ctg tac gac	912
Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp	
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ccc gtg ggt ggt gaa cac gcc aac tac ttc gcc gcc ggc ctg aag atg	960
Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met	
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Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys	
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Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp	
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Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn	
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ccc gag gtg gac gcc cac ctc aag tcg gac ggc tac acc aac ttc tcc	1152
Pro Glu Val Asp Ala His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser	
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ctg agg acg ctg gac tcc ggc aag cgg cag tgc aag gag gcc ctg cag	1200
Leu Arg Thr Leu Asp Ser Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln	
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cgc gag ctg ggc ctg cag gtc cgc gcc gac gtg ccg ctg ctc ggc ttc	1248
Arg Glu Leu Gly Leu Gln Val Arg Ala Asp Val Pro Leu Leu Gly Phe	
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 Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu Gly Thr  
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 Gly Arg His Asp Leu Glu Ser Met Leu Gln His Phe Glu Arg Glu His  
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 His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu Ala His  
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 Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg Phe Val  
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 Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro  
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 Val Val His Ala Val Gly Gly Leu Arg Asp Thr Val Pro Pro Phe Asp  
 515 520 525  
 ccc ttc aac cac tcc ggg ctc ggg tgg acg ttc gac cgc gcc gag gcg 1632  
 Pro Phe Asn His Ser Gly Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala  
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 His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp  
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 Phe Lys Glu Ser Trp Arg Ala Leu Gln Glu Arg Gly Met Ser Gln Asp  
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 Phe Ser Trp Glu His Ala Ala Lys Leu Tyr Glu Asp Val Leu Val Lys  
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 Ala Lys Tyr Gln Trp  
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Lys Lys Gly Ala Val Val Val Glu Glu Ala Pro Lys Pro Lys Ala Leu	35	40	45
Ser Pro Pro Ala Ala Pro Ala Val Gln Glu Asp Leu Trp Asp Phe Lys	50	55	60
Lys Tyr Ile Gly Phe Glu Glu Pro Val Glu Ala Lys Asp Asp Gly Arg	65	70	75
Ala Val Ala Asp Asp Ala Gly Ser Phe Glu His His Gln Asn His Asp	85	90	95
Ser Gly Pro Leu Ala Gly Glu Asn Val Met Asn Val Val Val Val Ala	100	105	110
Ala Glu Cys Ser Pro Trp Cys Lys Thr Gly Gly Leu Gly Asp Val Ala	115	120	125
Gly Ala Leu Pro Lys Ala Leu Ala Lys Arg Gly His Arg Val Met Val	130	135	140
Val Val Pro Arg Tyr Gly Asp Tyr Glu Glu Pro Thr Asp Val Gly Val	145	150	155
Arg Lys Tyr Tyr Lys Ala Ala Gly Gln Asp Met Glu Val Asn Tyr Phe	165	170	175
His Ala Tyr Ile Asp Gly Val Asp Phe Val Phe Ile Asp Ala Pro Leu	180	185	190
Phe Arg His Arg Glu Glu Asp Ile Tyr Gly Gly Ser Arg Gln Glu Ile	195	200	205
Met Lys Arg Met Ile Leu Phe Cys Lys Ala Ala Val Glu Val Pro Trp	210	215	220
His Val Pro Cys Gly Gly Val Pro Tyr Gly Asp Gly Asn Leu Val Phe	225	230	235
Ile Ala Asn Asp Trp His Thr Ala Leu Leu Pro Val Tyr Leu Lys Ala	245	250	255
Tyr Tyr Arg Asp His Gly Leu Met Gln Tyr Thr Arg Ser Ile Met Val	260	265	270
Ile His Asn Ile Ala His Gln Gly Arg Gly Pro Val Asp Glu Phe Pro	275	280	285
Phe Thr Glu Leu Pro Glu His Tyr Leu Glu His Phe Arg Leu Tyr Asp	290	295	300
Pro Val Gly Gly Glu His Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met	305	310	315
Ala Asp Gln Val Val Val Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys	325	330	335
Thr Val Glu Gly Gly Trp Gly Leu His Asp Ile Ile Arg Gln Asn Asp	340	345	350

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Trp Lys Thr Arg Gly Ile Val Asn Gly Ile Asp Asn Met Glu Trp Asn  
 355 360 365  
 Pro Glu Val Asp Ala His Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser  
 370 375 380  
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 Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Glu Ile Ile Ala Asp Ala  
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 Met Pro Trp Ile Val Ser Gln Asp Val Gln Leu Val Met Leu Gly Thr  
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 Gly Arg His Asp Leu Glu Ser Met Leu Gln His Phe Glu Arg Glu His  
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 His Asp Lys Val Arg Gly Trp Val Gly Phe Ser Val Arg Leu Ala His  
 465 470 475 480  
 Arg Ile Thr Ala Gly Ala Asp Ala Leu Leu Met Pro Ser Arg Phe Val  
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 Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro  
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 Val Val His Ala Val Gly Gly Leu Arg Asp Thr Val Pro Pro Phe Asp  
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 His Lys Leu Ile Glu Ala Leu Gly His Cys Leu Arg Thr Tyr Arg Asp  
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 Phe Lys Glu Ser Trp Arg Ala Leu Gln Glu Arg Gly Met Ser Gln Asp  
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 Ser Pro Leu Cys Pro Arg Ser Arg Gln Pro Leu Val Val Val Arg Pro

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10	15	20	
gcc ggc cgc ggc ggc ctc acg cag cct ttt ttg atg aat ggc aga ttt Ala Gly Arg Gly Gly Leu Thr Gln Pro Phe Leu Met Asn Gly Arg Phe 25 30 35 40			148
act cga agc agg acc ctt cga tgc atg gta gca agt tca gat cct cct Thr Arg Ser Arg Thr Leu Arg Cys Met Val Ala Ser Ser Asp Pro Pro 45 50 55			196
aat agg aaa tca aga agg atg gta cca cct cag gtt aaa gtc att tct Asn Arg Lys Ser Arg Arg Met Val Pro Pro Gln Val Lys Val Ile Ser 60 65 70			244
tct aga gga tat acg aca aga ctc att gtt gaa cca agc aac gag aat Ser Arg Gly Tyr Thr Thr Arg Leu Ile Val Glu Pro Ser Asn Glu Asn 75 80 85			292
aca gaa cac aat aat cgg gat gaa gaa act ctt gat aca tac aat gcg Thr Glu His Asn Asn Arg Asp Glu Glu Thr Leu Asp Thr Tyr Asn Ala 90 95 100			340
cta tta agt acc gag aca gca gaa tgg aca gat aat aga gaa gcc gag Leu Leu Ser Thr Glu Thr Ala Glu Trp Thr Asp Asn Arg Glu Ala Glu 105 110 115 120			388
act gct aaa gcg gac tcg tcg caa aat gct tta agc agt tct ata att Thr Ala Lys Ala Asp Ser Ser Gln Asn Ala Leu Ser Ser Ser Ile Ile 125 130 135			436
ggg gaa gtg gat gtg gcg gat gaa gat ata ctt gcg gct gat ctg aca Gly Glu Val Asp Val Ala Asp Glu Asp Ile Leu Ala Ala Asp Leu Thr 140 145 150			484
gtg tat tca ttg agc agt gta atg aag aag gaa gtg gat gca gcg gac Val Tyr Ser Leu Ser Ser Val Met Lys Lys Glu Val Asp Ala Ala Asp 155 160 165			532
aaa gct aga gtt aaa gaa gac gca ttt gag ctg gat ttg cca gca act Lys Ala Arg Val Lys Glu Asp Ala Phe Glu Leu Asp Leu Pro Ala Thr 170 175 180			580
aca ttg aga agt gtg ata gta gat gtg atg gat cat aat ggg act gta Thr Leu Arg Ser Val Ile Val Asp Val Met Asp His Asn Gly Thr Val 185 190 195 200			628
caa gag aca ttg aga agt gtg ata gta gat gtg atg gat cat aat ggg Gln Glu Thr Leu Arg Ser Val Ile Val Asp Val Met Asp His Asn Gly 205 210 215			676
act gta caa gag aca ttg aga agt gtg ata gta gat gtg atg gat gat Thr Val Gln Glu Thr Leu Arg Ser Val Ile Val Asp Val Met Asp Asp 220 225 230			724
gcg gcg gac aaa gct aga gtt gaa gaa gac gta ttt gag ctg gat ttg Ala Ala Asp Lys Ala Arg Val Glu Glu Asp Val Phe Glu Leu Asp Leu 235 240 245			772
tca gga aat att tca agc agt gcg acg acc gtg gaa cta gat gcg gtt Ser Gly Asn Ile Ser Ser Ser Ala Thr Thr Val Glu Leu Asp Ala Val 250 255 260			820
gac gaa gtc ggg cct gtt caa gac aaa ttt gag gcg acc tca tca gga Asp Glu Val Gly Pro Val Gln Asp Lys Phe Glu Ala Thr Ser Ser Gly 265 270 275 280			868



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agt caa gat ctt tcg gct gtt agt ctc cct aaa caa aac gta cca att Ser Gln Asp Leu Ser Ala Val Ser Leu Pro Lys Gln Asn Val Pro Ile 540 545 550	1684
gtt ggt acg tcg aga gag ggt caa aca aag caa gtt cct gtt gtt gat Val Gly Thr Ser Arg Glu Gly Gln Thr Lys Gln Val Pro Val Val Asp 555 560 565	1732
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cac aca tcc gag aaa act gat gag gat gcg ctt cat gta aag ttt aat His Thr Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn 585 590 595 600	1828
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tta att gag gat gat gga caa tat gaa gtt gac gag acc tct gtg tcc Leu Ile Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser 685 690 695	2116
gtt aac gtt gaa caa gat atc cag ggg tca cca cag gat gtt gtg gat Val Asn Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp 700 705 710	2164
ccg caa gca cta aag gtg atg ctg caa gaa ctc gct gag aaa aat tat Pro Gln Ala Leu Lys Val Met Leu Gln Glu Leu Ala Glu Lys Asn Tyr 715 720 725	2212
tcg atg agg aac aag ctg ttt gtt ttt cca gag gta gtg aaa gct gat Ser Met Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp 730 735 740	2260
tca gtt att gat ctt tat tta aat cgt gac cta aca gct ttg gcg aat Ser Val Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn 745 750 755 760	2308
gaa ccc gat gtc gtc atc aaa gga gca ttc aat ggt tgg aaa tgg agg Glu Pro Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg 765 770 775	2356
ctt ttc act gaa aga ttg cac aag agt gac ctt gga ggg gtt tgg tgg Leu Phe Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp 780 785 790	2404
tct tgc aaa ctg tac ata ccc aag gag gcc tac aga tta gac ttt gtg	2452

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Asn Val Ser Asn Ser Ala Thr Val Arg Glu Val Asp Ala Ser Asp Glu	
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gct ggg aat gat caa ggc ata ttt aga gca gat ttg tca gga aat gtt	964
Ala Gly Asn Asp Gln Gly Ile Phe Arg Ala Asp Leu Ser Gly Asn Val	
300 305 310	
ttt tca agc agt aca aca gtg gaa gtg ggt gca gtg gat gaa gct ggg	1012
Phe Ser Ser Ser Thr Thr Val Glu Val Gly Ala Val Asp Glu Ala Gly	
315 320 325	
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Ser Ile Lys Asp Arg Phe Glu Thr Asp Ser Ser Gly Asn Val Ser Thr	
330 335 340	
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Ser Ala Pro Met Trp Asp Ala Ile Asp Glu Thr Val Ala Asp Gln Asp	
345 350 355 360	
aca ttt gag gcg gat ttg tcg gga aat gct tca agc tgc gca aca tac	1156
Thr Phe Glu Ala Asp Leu Ser Gly Asn Ala Ser Ser Cys Ala Thr Tyr	
365 370 375	
aga gaa gtg gat gat gtg gtg gat gaa act aga tca gaa gag gaa aca	1204
Arg Glu Val Asp Asp Val Val Asp Glu Thr Arg Ser Glu Glu Glu Thr	
380 385 390	
ttt gca atg gat ttg ttt gca agt gaa tca ggc cat gag aaa cat atg	1252
Phe Ala Met Asp Leu Phe Ala Ser Glu Ser Gly His Glu Lys His Met	
395 400 405	
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Ala Val Asp Tyr Val Gly Glu Ala Thr Asp Glu Glu Glu Thr Tyr Gln	
410 415 420	
cag caa tat cca gta ccg tct tca ttc tct atg tgg gac aag gct att	1348
Gln Gln Tyr Pro Val Pro Ser Ser Phe Ser Met Trp Asp Lys Ala Ile	
425 430 435 440	
gct aaa aca ggt gta agt ttg aat cct gag ctg cga ctt gtc agg gtt	1396
Ala Lys Thr Gly Val Ser Leu Asn Pro Glu Leu Arg Leu Val Arg Val	
445 450 455	
gaa gaa caa ggc aaa gta aat ttt agt gat aaa aaa gac ctg tca att	1444
Glu Glu Gln Gly Lys Val Asn Phe Ser Asp Lys Lys Asp Leu Ser Ile	
460 465 470	
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Asp Asp Leu Pro Gly Gln Asn Gln Ser Ile Ile Gly Ser Tyr Lys Gln	
475 480 485	
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Asp Lys Ser Ile Ala Asp Val Ala Gly Pro Thr Gln Ser Ile Phe Gly	
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Ser Ser Lys Gln His Arg Ser Ile Val Ala Phe Pro Lys Gln Asn Gln	
505 510 515 520	
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Ser Ile Val Ser Val Thr Glu Gln Lys Gln Ser Ile Val Gly Phe Arg	
525 530 535	

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Leu Leu Ser Gln Lys His Ile Val Tyr Thr Glu Pro Leu Glu Ile Arg			
1065	. 1070	1075	1080
gcc gga acc aca gtg gat gtg cta tac aat ccc tct aac aca gtg cta			3316
Ala Gly Thr Thr Val Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu			
	1085	1090	1095
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Asn Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met			
	1100	1105	1110
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His Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp			
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Gly Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met			
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Met Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn			
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Arg Asn Gly Met Asp Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr			
	1165	1170	1175
gag aat tac atg cgt att atc cac att gcc gtt gag atg gcc ccc gtt			3604
Glu Asn Tyr Met Arg Ile Ile His Ile Ala Val Glu Met Ala Pro Val			
	1180	1185	1190
gca aag gtt gga ggt ctt ggg gat gtt gtt aca agt ctt tca cgt gcc			3652
Ala Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala			
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Cys Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe			
	1225	1230	1235
tct tgg ggt ggt aca gaa ata aaa gta tgg gtt gga cga gtc gaa gac			3796
Ser Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp			
	1245	1250	1255
ctg acc gtt tac ttc ctg gaa cct caa aat ggg atg ttt ggc gtt gga			3844
Leu Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly			
	1260	1265	1270
tgt gta tat gga agg aat gat gac cgc aga ttt ggg ttc ttc tgt cat			3892
Cys Val Tyr Gly Arg Asn Asp Arg Arg Phe Gly Phe Phe Cys His			
	1275	1280	1285
tct gct cta gag ttt atc ctc cag aat gaa ttt tct cca cat ata ata			3940
Ser Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile			
	1290	1295	1300
cat tgc cat gat tgg tca agt gct ccg gtc gcc tgg cta tat aag gaa			3988
His Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu			
	1305	1310	1315
			1320

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Ser	Cys	Lys	Leu	Tyr	Ile	Pro	Lys	Glu	Ala	Tyr	Arg	Leu	Asp	Phe	Val	
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Phe	Phe	Asn	Gly	Arg	Thr	Val	Tyr	Glu	Asn	Asn	Gly	Asn	Asn	Asp	Phe	
	810					815					820					
tgt	ata	gga	ata	gaa	ggc	act	atg	aat	gaa	gat	ctg	ttt	gag	gat	ttc	2548
Cys	Ile	Gly	Ile	Glu	Gly	Thr	Met	Asn	Glu	Asp	Leu	Phe	Glu	Asp	Phe	
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Arg	Ala	Ala	Asp	Glu	Ala	Val	Arg	Ala	Gln	Ala	Lys	Ala	Glu	Ile	Glu	
		875					880					885				
atc	aag	aag	aaa	aaa	ttg	caa	agt	atg	ttg	agt	ttg	gcc	aga	aca	tgt	2740
Ile	Lys	Lys	Lys	Lys	Leu	Gln	Ser	Met	Leu	Ser	Leu	Ala	Arg	Thr	Cys	
	890					895					900					
gtt	gat	aat	ttg	tgg	tac	ata	gag	gct	agc	aca	gat	aca	aga	gga	gat	2788
Val	Asp	Asn	Leu	Trp	Tyr	Ile	Glu	Ala	Ser	Thr	Asp	Thr	Arg	Gly	Asp	
905					910				915					920		
act	atc	agg	tta	tat	tat	aac	aga	aac	tcg	agg	cca	ctt	gcg	cat	agt	2836
Thr	Ile	Arg	Leu	Tyr	Tyr	Asn	Arg	Asn	Ser	Arg	Pro	Leu	Ala	His	Ser	
				925					930					935		
act	gag	att	tgg	atg	cat	ggt	ggt	tac	aac	aat	tgg	aca	gat	gga	ctc	2884
Thr	Glu	Ile	Trp	Met	His	Gly	Gly	Tyr	Asn	Asn	Trp	Thr	Asp	Gly	Leu	
			940					945					950			
tct	att	gtt	gaa	agc	ttt	gtc	aag	tgc	aat	gac	aaa	gac	ggc	gat	tgg	2932
Ser	Ile	Val	Glu	Ser	Phe	Val	Lys	Cys	Asn	Asp	Lys	Asp	Gly	Asp	Trp	
		955					960					965				
tgg	tat	gca	gat	gtt	att	cca	cct	gaa	aag	gca	ctt	gtg	ttg	gac	tgg	2980
Trp	Tyr	Ala	Asp	Val	Ile	Pro	Pro	Glu	Lys	Ala	Leu	Val	Leu	Asp	Trp	
	970					975					980					
gtt	ttt	gct	gat	ggg	cca	gct	ggg	aat	gca	agg	aac	tat	gac	aac	aat	3028
Val	Phe	Ala	Asp	Gly	Pro	Ala	Gly	Asn	Ala	Arg	Asn	Tyr	Asp	Asn	Asn	
985					990				995						1000	
gct	cga	caa	gat	ttc	cat	gct	att	ctt	ccg	aac	aac	aat	gta	acc	gag	3076
Ala	Arg	Gln	Asp	Phe	His	Ala	Ile	Leu	Pro	Asn	Asn	Asn	Val	Thr	Glu	
				1005					1010					1015		
gaa	ggc	ttc	tgg	gcg	caa	gag	gag	caa	aac	atc	tat	aca	agg	ctt	ctg	3124
Glu	Gly	Phe	Trp	Ala	Gln	Glu	Glu	Gln	Asn	Ile	Tyr	Thr	Arg	Leu	Leu	
			1020					1025					1030			
caa	gaa	agg	aga	gaa	aag	gaa	gaa	acc	atg	aaa	aga	aag	gct	gag	aga	3172
Gln	Glu	Arg	Arg	Glu	Lys	Glu	Glu	Thr	Met	Lys	Arg	Lys	Ala	Glu	Arg	
		1035					1040					1045				
agt	gca	aat	atc	aaa	gct	gag	atg	aag	gca	aaa	act	atg	cga	agg	ttt	3220
Ser	Ala	Asn	Ile	Lys	Ala	Glu	Met	Lys	Ala	Lys	Thr	Met	Arg	Arg	Phe	

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aat ggc gtg gat tat gcc ctc aac aga gca atc ggc gct tgg ttc gat 4804  
 Asn Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp  
                   1580                                  1585                                  1590

gcc cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac 4852  
 Ala Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp  
                   1595                                  1600                                  1605

tgg tcg tgg aac cgg ccc gca ctg gac tac att gaa ttg tac cat gcc 4900  
 Trp Ser Trp Asn Arg Pro Ala Leu Asp Tyr Ile Glu Leu Tyr His Ala  
                   1610                                  1615                                  1620

gct cga aaa ttc tgacacccaa ctgaaccaat gacaagaaca agcgcattgt 4952  
 Ala Arg Lys Phe  
 1625

gggatcgact agtcatacag ggctgtgcag atcgtcttgc ttcagttagt gccctcttca 5012  
 gttagtcca agcgactac agtcgtacat agctgaggat cctcttgcct cctaccaggg 5072  
 ggaacaaagc agaaatgcat gagtgcattg ggaagacttt tatgtatatt gttaaaaaaa 5132  
 tttccttttc tttccttcc ctgcacctgg aaatgggttaa gcgcacgcc gagataagaa 5192  
 ccgcagtgac attctgtgag tagctttgta tattctctca tcttgtgaaa actaatgttc 5252  
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                   20                                  25                                  30  
 Pro Phe Leu Met Asn Gly Arg Phe Thr Arg Ser Arg Thr Leu Arg Cys  
                   35                                  40                                  45  
 Met Val Ala Ser Ser Asp Pro Pro Asn Arg Lys Ser Arg Arg Met Val  
                   50                                  55                                  60  
 Pro Pro Gln Val Lys Val Ile Ser Ser Arg Gly Tyr Thr Thr Arg Leu  
                   65                                  70                                  75                                  80  
 Ile Val Glu Pro Ser Asn Glu Asn Thr Glu His Asn Asn Arg Asp Glu  
                   85                                  90                                  95  
 Glu Thr Leu Asp Thr Tyr Asn Ala Leu Leu Ser Thr Glu Thr Ala Glu  
                   100                                  105                                  110  
 Trp Thr Asp Asn Arg Glu Ala Glu Thr Ala Lys Ala Asp Ser Ser Gln  
                   115                                  120                                  125  
 Asn Ala Leu Ser Ser Ser Ile Ile Gly Glu Val Asp Val Ala Asp Glu  
                   130                                  135                                  140  
 Asp Ile Leu Ala Ala Asp Leu Thr Val Tyr Ser Leu Ser Ser Val Met

- 23 -

cac tat tcc caa tcc aga atg gca agc act cgg gtt gta ttt acc atc His Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile 1325 1330 1335	4036
cac aat ctt gaa ttt gga gca cat tat att ggt aaa gca atg aca tac His Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr 1340 1345 1350	4084
tgt gat aaa gcc aca act gtt tct cct aca tat tca agg gac gtg gca Cys Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala 1355 1360 1365	4132
ggc cat ggc gcc att gct cct cat cgt gag aaa ttc tac ggc att ctc Gly His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu 1370 1375 1380	4180
aat gga att gat cca gat atc tgg gat ccg tac act gac aat ttt atc Asn Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile 1385 1390 1395 1400	4228
ccg gtc cct tat act tgt gag aat gtt gtc gaa ggc aag aga gct gca Pro Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala 1405 1410 1415	4276
aaa agg gcc ttg cag cag aag ttt gga tta cag caa act gat gtc cct Lys Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro 1420 1425 1430	4324
att gtc gga atc atc acc cgt ctg aca gcc cag aag gga atc cac ctc Ile Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu 1435 1440 1445	4372
atc aag cac gca att cac cga act ctc gaa agc aac gga cat gtg gtt Ile Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly His Val Val 1450 1455 1460	4420
ttg ctt ggt tca gct cca gat cat cga ata caa ggc gat ttt tgc aga Leu Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg 1465 1470 1475 1480	4468
ttg gcc gat gct ctt cat ggt gtt tac cat ggt agg gtg aag ctt gtt Leu Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val 1485 1490 1495	4516
cta acc tat gat gag cct ctt tct cac ctg ata tac gct ggc tcg gac Leu Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ser Asp 1500 1505 1510	4564
ttc ata att gtt cct tca atc ttc gaa ccc tgt ggc tta aca caa ctt Phe Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu 1515 1520 1525	4612
gtt gcc atg cgt tat gga tcg atc cct ata gtt cgg aaa act gga gga Val Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly 1530 1535 1540	4660
ctt cac gac aca gtc ttc gac gta gac aat gat aag gac cgg gct cgg Leu His Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg 1545 1550 1555 1560	4708
tct ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc Ser Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser 1565 1570 1575	4756



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Gly Pro Thr Gln Ser Ile Phe Gly Ser Ser Lys Gln His Arg Ser Ile  
 500 505 510  
 Val Ala Phe Pro Lys Gln Asn Gln Ser Ile Val Ser Val Thr Glu Gln  
 515 520 525  
 Lys Gln Ser Ile Val Gly Phe Arg Ser Gln Asp Leu Ser Ala Val Ser  
 530 535 540  
 Leu Pro Lys Gln Asn Val Pro Ile Val Gly Thr Ser Arg Glu Gly Gln  
 545 550 555 560  
 Thr Lys Gln Val Pro Val Val Asp Arg Gln Asp Ala Leu Tyr Val Asn  
 565 570 575  
 Gly Leu Glu Ala Lys Glu Gly Asp His Thr Ser Glu Lys Thr Asp Glu  
 580 585 590  
 Asp Ala Leu His Val Lys Phe Asn Val Asp Asn Val Leu Arg Lys His  
 595 600 605  
 Gln Ala Asp Arg Thr Gln Ala Val Glu Lys Lys Thr Trp Lys Lys Val  
 610 615 620  
 Asp Glu Glu His Leu Tyr Met Thr Glu His Gln Lys Arg Ala Ala Glu  
 625 630 635 640  
 Gly Gln Met Val Val Asn Glu Asp Glu Leu Ser Ile Thr Glu Ile Gly  
 645 650 655  
 Met Gly Arg Gly Asp Lys Ile Gln His Val Leu Ser Glu Glu Glu Leu  
 660 665 670  
 Ser Trp Ser Glu Asp Glu Val Gln Leu Ile Glu Asp Asp Gly Gln Tyr  
 675 680 685  
 Glu Val Asp Glu Thr Ser Val Ser Val Asn Val Glu Gln Asp Ile Gln  
 690 695 700  
 Gly Ser Pro Gln Asp Val Val Asp Pro Gln Ala Leu Lys Val Met Leu  
 705 710 715 720  
 Gln Glu Leu Ala Glu Lys Asn Tyr Ser Met Arg Asn Lys Leu Phe Val  
 725 730 735  
 Phe Pro Glu Val Val Lys Ala Asp Ser Val Ile Asp Leu Tyr Leu Asn  
 740 745 750  
 Arg Asp Leu Thr Ala Leu Ala Asn Glu Pro Asp Val Val Ile Lys Gly  
 755 760 765  
 Ala Phe Asn Gly Trp Lys Trp Arg Leu Phe Thr Glu Arg Leu His Lys  
 770 775 780  
 Ser Asp Leu Gly Gly Val Trp Trp Ser Cys Lys Leu Tyr Ile Pro Lys  
 785 790 795 800  
 Glu Ala Tyr Arg Leu Asp Phe Val Phe Phe Asn Gly Arg Thr Val Tyr  
 805 810 815  
 Glu Asn Asn Gly Asn Asn Asp Phe Cys Ile Gly Ile Glu Gly Thr Met  
 820 825 830  
 Asn Glu Asp Leu Phe Glu Asp Phe Leu Val Lys Glu Lys Gln Arg Glu  
 835 840 845



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145		150		155		160
Lys Lys Glu Val Asp	Ala Ala Asp	Lys Ala Arg	Val Lys Glu Asp	Ala		
	165		170		175	
Phe Glu Leu Asp Leu Pro	Ala Thr Thr	Leu Arg Ser	Val Ile Val Asp			
	180	185	190			
Val Met Asp His Asn Gly Thr	Val Gln Glu Thr	Leu Arg Ser	Val Ile			
	195	200	205			
Val Asp Val Met Asp His	Asn Gly Thr	Val Gln Glu Thr	Leu Arg Ser			
	210	215	220			
Val Ile Val Asp Val Met	Asp Asp Ala Ala	Asp Lys Ala Arg	Val Glu			
	225	230	235		240	
Glu Asp Val Phe Glu Leu	Asp Leu Ser Gly	Asn Ile Ser Ser	Ser Ala			
	245	250	255			
Thr Thr Val Glu Leu Asp	Ala Val Asp	Glu Val Gly Pro	Val Gln Asp			
	260	265	270			
Lys Phe Glu Ala Thr Ser	Ser Gly Asn Val	Ser Asn Ser	Ala Thr Val			
	275	280	285			
Arg Glu Val Asp Ala Ser	Asp Glu Ala Gly	Asn Asp Gln Gly	Ile Phe			
	290	295	300			
Arg Ala Asp Leu Ser Gly	Asn Val Phe Ser	Ser Ser Thr Thr	Val Glu			
	305	310	315		320	
Val Gly Ala Val Asp Glu	Ala Gly Ser Ile	Lys Asp Arg Phe	Glu Thr			
	325	330	335			
Asp Ser Ser Gly Asn Val	Ser Thr Ser	Ala Pro Met Trp	Asp Ala Ile			
	340	345	350			
Asp Glu Thr Val Ala Asp	Gln Asp Thr Phe	Glu Ala Asp	Leu Ser Gly			
	355	360	365			
Asn Ala Ser Ser Cys Ala	Thr Tyr Arg Glu	Val Asp Asp	Val Val Asp			
	370	375	380			
Glu Thr Arg Ser Glu Glu	Glu Thr Phe Ala	Met Asp Leu Phe	Ala Ser			
	385	390	395		400	
Glu Ser Gly His Glu Lys	His Met Ala Val	Asp Tyr Val Gly	Glu Ala			
	405	410	415			
Thr Asp Glu Glu Glu Thr	Tyr Gln Gln Gln	Tyr Pro Val Pro	Ser Ser			
	420	425	430			
Phe Ser Met Trp Asp Lys	Ala Ile Ala Lys	Thr Gly Val Ser	Leu Asn			
	435	440	445			
Pro Glu Leu Arg Leu Val	Arg Val Glu Glu	Gln Gly Lys Val	Asn Phe			
	450	455	460			
Ser Asp Lys Lys Asp Leu	Ser Ile Asp Asp	Leu Pro Gly Gln	Asn Gln			
	465	470	475		480	
Ser Ile Ile Gly Ser Tyr	Lys Gln Asp Lys	Ser Ile Ala Asp	Val Ala			
	485	490	495			

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185                      1190                      1195                      1200  
 Val Val Thr Ser Leu Ser Arg Ala Ile Gln Asp Leu Gly His Thr Val  
                          1205                      1210                      1215  
 Glu Val Ile Leu Pro Lys Tyr Asp Cys Leu Asn Gln Ser Ser Val Lys  
                          1220                      1225                      1230  
 Asp Leu His Leu Tyr Gln Ser Phe Ser Trp Gly Gly Thr Glu Ile Lys  
                          1235                      1240                      1245  
 Val Trp Val Gly Arg Val Glu Asp Leu Thr Val Tyr Phe Leu Glu Pro  
                          1250                      1255                      1260  
 Gln Asn Gly Met Phe Gly Val Gly Cys Val Tyr Gly Arg Asn Asp Asp  
 265                      1270                      1275                      1280  
 Arg Arg Phe Gly Phe Phe Cys His Ser Ala Leu Glu Phe Ile Leu Gln  
                          1285                      1290                      1295  
 Asn Glu Phe Ser Pro His Ile Ile His Cys His Asp Trp Ser Ser Ala  
                          1300                      1305                      1310  
 Pro Val Ala Trp Leu Tyr Lys Glu His Tyr Ser Gln Ser Arg Met Ala  
                          1315                      1320                      1325  
 Ser Thr Arg Val Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala His  
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 Tyr Ile Gly Lys Ala Met Thr Tyr Cys Asp Lys Ala Thr Thr Val Ser  
 345                      1350                      1355                      1360  
 Pro Thr Tyr Ser Arg Asp Val Ala Gly His Gly Ala Ile Ala Pro His  
                          1365                      1370                      1375  
 Arg Glu Lys Phe Tyr Gly Ile Leu Asn Gly Ile Asp Pro Asp Ile Trp  
                          1380                      1385                      1390  
 Asp Pro Tyr Thr Asp Asn Phe Ile Pro Val Pro Tyr Thr Cys Glu Asn  
                          1395                      1400                      1405  
 Val Val Glu Gly Lys Arg Ala Ala Lys Arg Ala Leu Gln Gln Lys Phe  
                          1410                      1415                      1420  
 Gly Leu Gln Gln Thr Asp Val Pro Ile Val Gly Ile Ile Thr Arg Leu  
 425                      1430                      1435                      1440  
 Thr Ala Gln Lys Gly Ile His Leu Ile Lys His Ala Ile His Arg Thr  
                          1445                      1450                      1455  
 Leu Glu Ser Asn Gly His Val Val Leu Leu Gly Ser Ala Pro Asp His  
                          1460                      1465                      1470  
 Arg Ile Gln Gly Asp Phe Cys Arg Leu Ala Asp Ala Leu His Gly Val  
                          1475                      1480                      1485  
 Tyr His Gly Arg Val Lys Leu Val Leu Thr Tyr Asp Glu Pro Leu Ser  
                          1490                      1495                      1500  
 His Leu Ile Tyr Ala Gly Ser Asp Phe Ile Ile Val Pro Ser Ile Phe  
 505                      1510                      1515                      1520  
 Glu Pro Cys Gly Leu Thr Gln Leu Val Ala Met Arg Tyr Gly Ser Ile  
                          1525                      1530                      1535

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Leu Glu Lys Leu Ala Met Glu Glu Ala Glu Arg Arg Thr Gln Thr Glu  
 850 855 860  
 Glu Gln Arg Arg Arg Lys Glu Ala Arg Ala Ala Asp Glu Ala Val Arg  
 865 870 875 880  
 Ala Gln Ala Lys Ala Glu Ile Glu Ile Lys Lys Lys Lys Leu Gln Ser  
 885 890 895  
 Met Leu Ser Leu Ala Arg Thr Cys Val Asp Asn Leu Trp Tyr Ile Glu  
 900 905 910  
 Ala Ser Thr Asp Thr Arg Gly Asp Thr Ile Arg Leu Tyr Tyr Asn Arg  
 915 920 925  
 Asn Ser Arg Pro Leu Ala His Ser Thr Glu Ile Trp Met His Gly Gly  
 930 935 940  
 Tyr Asn Asn Trp Thr Asp Gly Leu Ser Ile Val Glu Ser Phe Val Lys  
 945 950 955 960  
 Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr Ala Asp Val Ile Pro Pro  
 965 970 975  
 Glu Lys Ala Leu Val Leu Asp Trp Val Phe Ala Asp Gly Pro Ala Gly  
 980 985 990  
 Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg Gln Asp Phe His Ala Ile  
 995 1000 1005  
 Leu Pro Asn Asn Asn Val Thr Glu Glu Gly Phe Trp Ala Gln Glu Glu  
 1010 1015 1020  
 Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu Arg Arg Glu Lys Glu Glu  
 1025 1030 1035 1040  
 Thr Met Lys Arg Lys Ala Glu Arg Ser Ala Asn Ile Lys Ala Glu Met  
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 Lys Ala Lys Thr Met Arg Arg Phe Leu Leu Ser Gln Lys His Ile Val  
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 Tyr Thr Glu Pro Leu Glu Ile Arg Ala Gly Thr Thr Val Asp Val Leu  
 1075 1080 1085  
 Tyr Asn Pro Ser Asn Thr Val Leu Asn Gly Lys Ser Glu Gly Trp Phe  
 1090 1095 1100  
 Arg Cys Ser Phe Asn Leu Trp Met His Ser Ser Gly Ala Leu Pro Pro  
 1105 1110 1115 1120  
 Gln Lys Met Val Lys Ser Gly Asp Gly Pro Leu Leu Lys Ala Thr Val  
 1125 1130 1135  
 Asp Val Pro Pro Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu Trp  
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 Glu Glu Asp Gly Ile Tyr Asp Asn Arg Asn Gly Met Asp Tyr His Ile  
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 Pro Val Ser Asp Ser Ile Glu Thr Glu Asn Tyr Met Arg Ile Ile His  
 1170 1175 1180  
 Ile Ala Val Glu Met Ala Pro Val Ala Lys Val Gly Gly Leu Gly Asp

- 30 -

gca cta aag gtg atg ctg caa gaa ctc gct gag aaa aat tat tcg atg	480
Ala Leu Lys Val Met Leu Gln Glu Leu Ala Glu Lys Asn Tyr Ser Met	
145 150 155 160	
agg aac aag ctg ttt gtt ttt cca gag gta gtg aaa gct gat tca gtt	528
Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp Ser Val	
165 170 175	
att gat ctt tat tta aat cgt gac cta aca gct ttg gcg aat gaa ccc	576
Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn Glu Pro	
180 185 190	
gat gtc gtc atc aaa gga gca ttc aat ggt tgg aaa tgg agg ctt ttc	624
Asp Val Val Ile Lys Gly Ala Phe Asn Gly Trp Lys Trp Arg Leu Phe	
195 200 205	
act gaa aga ttg cac aag agt gac ctt gga ggg gtt tgg tgg tct tgc	672
Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp Ser Cys	
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Lys Leu Tyr Ile Pro Lys Glu Ala Tyr Arg Leu Asp Phe Val Phe Phe	
225 230 235 240	
aac ggt cgc acg gtc tat gag aac aat ggc aac aat gat ttc tgt ata	768
Asn Gly Arg Thr Val Tyr Glu Asn Asn Gly Asn Asn Asp Phe Cys Ile	
245 250 255	
gga ata gaa ggc act atg aat gaa gat ctg ttt gag gat ttc ttg gtt	816
Gly Ile Glu Gly Thr Met Asn Glu Asp Leu Phe Glu Asp Phe Leu Val	
260 265 270	
aaa gaa aag caa agg gag ctt gag aaa ctt gcc atg gaa gaa gct gaa	864
Lys Glu Lys Gln Arg Glu Leu Glu Lys Leu Ala Met Glu Glu Ala Glu	
275 280 285	
agg agg aca cag act gaa gaa cag cgg cga aga aag gaa gca agg gct	912
Arg Arg Thr Gln Thr Glu Glu Gln Arg Arg Arg Lys Glu Ala Arg Ala	
290 295 300	
gca gat gaa gct gtc agg gca caa gcg aag gcc gag ata gag atc aag	960
Ala Asp Glu Ala Val Arg Ala Gln Ala Lys Ala Glu Ile Glu Ile Lys	
305 310 315 320	
aag aaa aaa ttg caa agt atg ttg agt ttg gcc aga aca tgt gtt gat	1008
Lys Lys Lys Leu Gln Ser Met Leu Ser Leu Ala Arg Thr Cys Val Asp	
325 330 335	
aat ttg tgg tac ata gag gct agc aca gat aca aga gga gat act atc	1056
Asn Leu Trp Tyr Ile Glu Ala Ser Thr Asp Thr Arg Gly Asp Thr Ile	
340 345 350	
agg tta tat tat aac aga aac tcg agg cca ctt gcg cat agt act gag	1104
Arg Leu Tyr Tyr Asn Arg Asn Ser Arg Pro Leu Ala His Ser Thr Glu	
355 360 365	
att tgg atg cat ggt ggt tac aac aat tgg tca gat gga ctc tct att	1152
Ile Trp Met His Gly Gly Tyr Asn Asn Trp Ser Asp Gly Leu Ser Ile	
370 375 380	
gtt gaa agc ttt gtc aag tgc aat gac aaa gac ggc gat tgg tgg tat	1200
Val Glu Ser Phe Val Lys Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr	
385 390 395 400	
gca gat gtt att cca cct gaa aag gca ctt gtg ttg gac tgg gtt ttt	1248

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Pro Ile Val Arg Lys Thr Gly Gly Leu His Asp Thr Val Phe Asp Val  
 1540 1545 1550

Asp Asn Asp Lys Asp Arg Ala Arg Ser Leu Gly Leu Glu Pro Asn Gly  
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Phe Ser Phe Asp Gly Ala Asp Ser Asn Gly Val Asp Tyr Ala Leu Asn  
 1570 1575 1580

Arg Ala Ile Gly Ala Trp Phe Asp Ala Arg Asp Trp Phe His Ser Leu  
 585 1590 1595 1600

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tcc gag aaa act gat gag gat gcg ctt cat gta aag ttt aat gtt gac 96  
 Ser Glu Lys Thr Asp Glu Asp Ala Leu His Val Lys Phe Asn Val Asp  
 20 25 30

aat gtg ttg cgg aag cat cag gca gat aga acc caa gca gtg gaa aag 144  
 Asn Val Leu Arg Lys His Gln Ala Asp Arg Thr Gln Ala Val Glu Lys  
 35 40 45

aaa act tgg aag aaa gtt gat gag gaa cat ctt tac atg act gaa cat 192  
 Lys Thr Trp Lys Lys Val Asp Glu Glu His Leu Tyr Met Thr Glu His  
 50 55 60

cag aaa cgt gct gcc gaa gga cag atg gta gtt aac gag gat gag ctt 240  
 Gln Lys Arg Ala Ala Glu Gly Gln Met Val Val Asn Glu Asp Glu Leu  
 65 70 75 80

tct ata act gaa att gga atg ggg aga ggt gat aaa att cag cat gtg 288  
 Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln His Val  
 85 90 95

ctt tct gag gaa gag ctt tca tgg tct gaa gat gaa gtg cag tta att 336  
 Leu Ser Glu Glu Glu Leu Ser Trp Ser Glu Asp Glu Val Gln Leu Ile  
 100 105 110

gag gat gat gga caa tat gaa gtt gac gag acc tct gtg tcc gtt aac 384  
 Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser Val Asn  
 115 120 125

gtt gaa caa gat atc cag ggg tca cca cag gat gtt gtg gat ccg caa 432  
 Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp Pro Gln  
 130 135 140

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660	665	670	
tgg ggt ggt aca gaa ata aaa gta tgg gtt gga cga gtc gaa gac ctg Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp Leu 675 680 685			2064
acc gtt tac ttc ctg gaa cct caa aat ggg atg ttt ggc gtt gga tgt Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly Cys 690 695 700			2112
gta tat gga agg aat gat gac cgc aga ttt ggg ttc ttc tgt cat tct Val Tyr Gly Arg Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His Ser 705 710 715 720			2160
gct cta gag ttt atc ctc cag aat gaa ttt tct cca cat ata ata cat Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile His 725 730 735			2208
tgc cat gat tgg tca agt gct ccg gtc gcc tgg cta tat aag gaa cac Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu His 740 745 750			2256
tat tcc caa tcc aga atg gca agc act cgg gtt gta ttt acc atc cac Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile His 755 760 765			2304
aat ctt gaa ttt gga gca cat tat att ggt aaa gca atg aca tac tgt Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr Cys 770 775 780			2352
gat aaa gcc aca act gtt tct cct aca tat tca agg gac gtg gca ggc Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala Gly 785 790 795 800			2400
cat ggc gcc att gct cct cat cgt gag aaa ttc tac ggc att ctc aat His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu Asn 805 810 815			2448
gga att gat cca gat atc tgg gat ccg tac act gac aat ttt atc ccg Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile Pro 820 825 830			2496
gtc cct tat act tgt gag aat gtt gtc gaa ggc aag agg gct gca aaa Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala Lys 835 840 845			2544
agg gcc ttg cag cag aag ttt gga tta cag caa act gat gtc cct att Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro Ile 850 855 860			2592
gtc gga atc atc acc cgt ctg aca gca cag aag gga atc cac ctc atc Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu Ile 865 870 875 880			2640
aag cac gca att cac cga acc ctc gag agc aat gga caa gtg gtt ttg Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly Gln Val Val Leu 885 890 895			2688
ctt ggt tca gct cca gat cat cga ata caa ggc gat ttt tgc aga ttg Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg Leu 900 905 910			2736
gcc gat gct ctt cac ggt gtt tac cat ggt agg gtg aag ctt gtt cta Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val Leu 915 920 925			2784

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Ala Asp Gly Pro Ala Gly Asn Ala Arg Asn Tyr Asp Asn Asn Ala Arg	
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caa gat ttc cat gct att ctt ccg aac aac aat gta acc gag gaa ggc	1344
Gln Asp Phe His Ala Ile Leu Pro Asn Asn Asn Val Thr Glu Glu Gly	
435 440 445	
ttc tgg gcg caa gag gag caa aac atc tat aca agg ctt ctg caa gaa	1392
Phe Trp Ala Gln Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu	
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Arg Arg Glu Lys Glu Glu Thr Met Lys Arg Lys Ala Glu Arg Ser Ala	
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Asn Ile Lys Ala Glu Met Lys Ala Lys Thr Met Arg Arg Phe Leu Leu	
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Ser Gln Lys His Ile Val Tyr Thr Arg Thr Xaa Leu Lys Tyr Val Pro	
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Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met His	
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Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp Gly	
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Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met Met	
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Asn Gly Met Asp Tyr His Ile Pro Val Ser Asp Ser Ile Glu Thr Glu	
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Asn Tyr Met Arg Ile Ile His Ile Ala Val Glu Met Ala Pro Val Ala	
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Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Ile	
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Gln Asp Leu Gly His Thr Val Glu Val Ile Leu Pro Lys Tyr Asp Cys	
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Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe Ser	



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 Ser Ile Thr Glu Ile Gly Met Gly Arg Gly Asp Lys Ile Gln His Val  
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 Leu Ser Glu Glu Glu Leu Ser Trp Ser Glu Asp Glu Val Gln Leu Ile  
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 Glu Asp Asp Gly Gln Tyr Glu Val Asp Glu Thr Ser Val Ser Val Asn  
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 Val Glu Gln Asp Ile Gln Gly Ser Pro Gln Asp Val Val Asp Pro Gln  
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 Arg Asn Lys Leu Phe Val Phe Pro Glu Val Val Lys Ala Asp Ser Val  
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 Ile Asp Leu Tyr Leu Asn Arg Asp Leu Thr Ala Leu Ala Asn Glu Pro  
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 Thr Glu Arg Leu His Lys Ser Asp Leu Gly Gly Val Trp Trp Ser Cys  
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 Lys Glu Lys Gln Arg Glu Leu Glu Lys Leu Ala Met Glu Glu Ala Glu  
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 Lys Lys Lys Leu Gln Ser Met Leu Ser Leu Ala Arg Thr Cys Val Asp  
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 Arg Leu Tyr Tyr Asn Arg Asn Ser Arg Pro Leu Ala His Ser Thr Glu  
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 Val Glu Ser Phe Val Lys Cys Asn Asp Lys Asp Gly Asp Trp Trp Tyr

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att att gtc cct tca atc ttt gaa ccc tgt ggc tta aca caa ctt gtt 2880
Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val
    945                      950                      955                      960

gcc atg cgt tat gga tcg atc cct ata gtt cgg aaa acc gga gga ctt 2928
Ala Met Arg Tyr Gly Ser Ile Pro Ile Val Arg Lys Thr Gly Gly Leu
                      965                      970                      975

tac gac act gtc ttc gac gta gac aat gat aag gac cgg gct cgg tct 2976
Tyr Asp Thr Val Phe Asp Val Asp Asn Asp Lys Asp Arg Ala Arg Ser
                      980                      985                      990

ctt ggt ctt gaa cca aat ggg ttc agt ttc gac gga gcc gac agc aat 3024
Leu Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp Gly Ala Asp Ser Asn
    995                      1000                      1005

ggc gtg gat tat gcc ctc aac aga gca atc ggc gct tgg ttc gat gcc 3072
Gly Val Asp Tyr Ala Leu Asn Arg Ala Ile Gly Ala Trp Phe Asp Ala
    1010                      1015                      1020

cgt gat tgg ttc cac tcc ctg tgt aag agg gtc atg gag caa gac tgg 3120
Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp
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Arg Lys Phe

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 35 40 45

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Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Tyr Lys Glu His  
 740 745 750  
 Tyr Ser Gln Ser Arg Met Ala Ser Thr Arg Val Val Phe Thr Ile His  
 755 760 765  
 Asn Leu Glu Phe Gly Ala His Tyr Ile Gly Lys Ala Met Thr Tyr Cys  
 770 775 780  
 Asp Lys Ala Thr Thr Val Ser Pro Thr Tyr Ser Arg Asp Val Ala Gly  
 785 790 795 800  
 His Gly Ala Ile Ala Pro His Arg Glu Lys Phe Tyr Gly Ile Leu Asn  
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 Gly Ile Asp Pro Asp Ile Trp Asp Pro Tyr Thr Asp Asn Phe Ile Pro  
 820 825 830  
 Val Pro Tyr Thr Cys Glu Asn Val Val Glu Gly Lys Arg Ala Ala Lys  
 835 840 845  
 Arg Ala Leu Gln Gln Lys Phe Gly Leu Gln Gln Thr Asp Val Pro Ile  
 850 855 860  
 Val Gly Ile Ile Thr Arg Leu Thr Ala Gln Lys Gly Ile His Leu Ile  
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 Lys His Ala Ile His Arg Thr Leu Glu Ser Asn Gly Gln Val Val Leu  
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 Leu Gly Ser Ala Pro Asp His Arg Ile Gln Gly Asp Phe Cys Arg Leu  
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 Ala Asp Ala Leu His Gly Val Tyr His Gly Arg Val Lys Leu Val Leu  
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 930 935 940  
 Ile Ile Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln Leu Val  
 945 950 955 960  
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 965 970 975  
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 980 985 990  
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 Arg Asp Trp Phe His Ser Leu Cys Lys Arg Val Met Glu Gln Asp Trp  
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Gln Asp Phe His Ala Ile Leu Pro Asn Asn Asn Val Thr Glu Glu Gly						
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Phe Trp Ala Gln Glu Glu Gln Asn Ile Tyr Thr Arg Leu Leu Gln Glu						
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Arg Arg Glu Lys Glu Glu Thr Met Lys Arg Lys Ala Glu Arg Ser Ala						
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Ser Gln Lys His Ile Val Tyr Thr Arg Thr Xaa Leu Lys Tyr Val Pro						
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Gly Thr Thr Val Asp Val Leu Tyr Asn Pro Ser Asn Thr Val Leu Asn						
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Gly Lys Ser Glu Gly Trp Phe Arg Cys Ser Phe Asn Leu Trp Met His						
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Ser Ser Gly Ala Leu Pro Pro Gln Lys Met Val Lys Ser Gly Asp Gly						
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Pro Leu Leu Lys Ala Thr Val Asp Val Pro Pro Asp Ala Tyr Met Met						
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Asp Phe Val Phe Ser Glu Trp Glu Glu Asp Gly Ile Tyr Asp Asn Arg						
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Asn Tyr Met Arg Ile Ile His Ile Ala Val Glu Met Ala Pro Val Ala						
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Lys Val Gly Gly Leu Gly Asp Val Val Thr Ser Leu Ser Arg Ala Ile						
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Gln Asp Leu Gly His Thr Val Glu Val Ile Leu Pro Lys Tyr Asp Cys						
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Leu Asn Gln Ser Ser Val Lys Asp Leu His Leu Tyr Gln Ser Phe Ser						
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Trp Gly Gly Thr Glu Ile Lys Val Trp Val Gly Arg Val Glu Asp Leu						
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Thr Val Tyr Phe Leu Glu Pro Gln Asn Gly Met Phe Gly Val Gly Cys						
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Val Tyr Gly Arg Asn Asp Asp Arg Arg Phe Gly Phe Phe Cys His Ser						
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Ala Leu Glu Phe Ile Leu Gln Asn Glu Phe Ser Pro His Ile Ile His						
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 <213> Triticum sp.

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<220>  
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<210> 21  
<211> 8  
<212> PRT  
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<220>  
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 21  
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1 5

<210> 22  
<211> 8  
<212> PRT  
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<220>  
<223> Description of Artificial Sequence:PEPTIDE MOTIF

<400> 22  
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<210> 23  
<211> 14  
<212> PRT  
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<220>  
<223> Description of Artificial Sequence:PEPTIDE MOTIF

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1 5 10

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<210> 17  
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<220>  
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<210> 18  
 <211> 10  
 <212> PRT  
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<220>  
 <223> Description of Artificial Sequence: PEPTIDE MOTIF

<400> 18  
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 1 5 10

<210> 19

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<220>  
<223> Description of Artificial Sequence:PRIMER

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<210> 30  
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<212> DNA  
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<223> Description of Artificial Sequence:PRIMER

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<210> 31  
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<400> 31  
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<223> Description of Artificial Sequence:PRIMER

<400> 32  
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<210> 33  
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<400> 33  
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<210> 34  
<211> 21  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence:PRIMER

<400> 34

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<210> 24  
<211> 13  
<212> PRT  
<213> Artificial Sequence

<220>  
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<400> 25  
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<210> 26  
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<212> DNA  
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<220>  
<223> Description of Artificial Sequence:PRIMER

<400> 26  
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<210> 27  
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<400> 27  
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<400> 28  
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21

<210> 35  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PEPTIDE MOTIF

<400> 35  
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<210> 36  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PEPTIDE MOTIF

<400> 36  
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 Gln Asp Leu Gly His Thr Val Glu Val  
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 <211> 11611  
 <212> DNA  
 <213> *Triticum aestivum*

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<210> 42

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- 58 -

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<210> 52  
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<220>  
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Leu Asn Gln Leu Tyr Ala Met Ala Tyr Gly Thr  
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<212> PRT  
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/00385

**A. CLASSIFICATION OF SUBJECT MATTER**Int. Cl. <sup>7</sup>: C12N 15/54, 15/11; C12N 9/10; C12Q 1/48, 1/68; A01H 1/00, 5/00; C08B 3/02.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**Minimum documentation searched (classification system followed by classification symbols)  
WORLD PATENT INDEX (WPI).Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
GENBANK, EMBL, SWISS-PROTEINS, PIRElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
KW: WPI Starch synthase. Seq id nos 2, 4, 6, 8, 10 and 39-54.**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	Li Z <i>et al</i> "The localization and expression of the class II starch synthases of wheat" Plant Physiol 1999 Aug 120(4) pp 1147-1156. See the whole document.	1-59.
P, X	GenPept accession no. CAB86618, and GenBank accession no. AJ269502, published 7 April 2000. Gao M and Chibbar R N "Isolation, characterization and expression analysis of starch synthase IIa c DNA from wheat ( <i>Triticum aestivum</i> L.)" See the whole document.	1-8, 10-19 and 21 (seq id nos 1-6, 50 and 53)
X; Y	WO 97/45545 A (HOECHST SCHERING AGREVO GmbH) 4 December 1997. See the whole document especially the examples and seq id no 5.	1-8, 10-19, 21-38 and 41-59 (seq id nos 1-6, 50 and 53)

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
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Date of the actual completion of the international search 16 June 2000	Date of mailing of the international search report 20 JUN 2000
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized officer  J.H. CHAN Telephone No : (02) 6283 2340



# INTERNATIONAL SEARCH REPORT

## Information on patent family members

International application No.  
PCT/AU00/00385

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	9745545	AU	30302/97	BR	9709487	CN	1219970
		CZ	9803890	DE	19621588	EP	907741
		SK	1636/98	ZA	9704657		
END OF ANNEX							